ISSN 00 22-09 81
JEMBAM 47(1) 1–100 (1980)
0, Volume 47, Number 1, pp. 1–100
Published 14 August 1980

JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

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JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

Harold Barnes, Founder Editor

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Papers on all aspects of marine ecology – floristic or faunistic, littoral, inshore, offshore, and deep sea – will be acceptable. The journal is particularly intended, however, to be a forum for work on the physiology, biochemistry, and behaviour of organisms in relation to their ecology; experimental work on eco-systems and on individual organisms in relation to their ecology will be especially welcome. Although this kind of work will be stressed, papers of a descriptive character – particularly if they aim at elucidating or illuminating general ecological problems and principles – will be considered for inclusion. Articles on new techniques and methods will, if their importance warrants it, be published, as will those concerned with 'theoretical' ecology. Every effort will be made to expedite publication.

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Subscriptions should be sent to the publishers, **Elsevier/North-Holland Biomedical Press, Journal Division, P.O. Box 211, 1000 AE Amsterdam, The Netherlands.**

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Library of Congress Catalog Card Number 68–26535

Printed in Belgium

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ELSEVIER/NORTH-HOLLAND BIOMEDICAL PRESS – AMSTERDAM

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PRINTED IN BELGIUM

J. exp. mar. Biol. Ecol., 1980, Vol. 47, pp. 1–8
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A POSSIBLE RÔLE OF α-AMYLASE ISOENZYMES FROM THE STYLE OF THE MUSSEL *CHOROMYTILUS MERIDIONALIS* (Krauss) FOLLOWING THERMAL ACCLIMATION

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Abstract: Separation of the proteins comprising the crystalline style of the mussel *Choromytilus meridionalis* (Krauss) by anion exchange chromatography shows that there are three fractions displaying α-amylase activity in both warm- and cold-acclimated mussels. These fractions correspond with one or more proteins which remain unbound to the resin (Peak I), a bound fraction which is eluted at 100–150 mM NaCl (Peak II) and a further fraction which is eluted at 200–250 mM NaCl (Peak III) but which may represent contamination carried over from Peak II. Cold-acclimation to 8 °C results in the appearance of a fourth α-amylase fraction (Peak IV) which is eluted from the column between 300–400 mM NaCl. Thermal acclimation also results in changes in the activities of Fractions I–IV such that a specific activity of 0.47 mg glucose liberated per A_{280} unit of protein per 8 min incubation at 8 °C in Fraction IV is increased nearly 10-fold to a specific rate of 4.10 in protein Fraction I following acclimation to 22 °C. It is suggested that an increase of digestive activity may be of equal importance to a suppression of metabolic costs in the maintenance of energy flow into growth and reproduction in ectothermic organisms which experience an increase of environmental temperature, especially in bivalves such as *C. meridionalis* which do not show a compensatory increase in filtration rate.

INTRODUCTION

Many marine molluscs are able to compensate for an increase in metabolic energy costs by an enhancement of filtration rate as environmental temperatures rise seasonally or along a latitudinal gradient (Newell & Kofoed, 1977a, b; Newell *et al.*, 1977). Others, such as the bivalves *Modiolus modiolus* and *Arctica islandica*, may show a compensatory increase in both absorption efficiency and filtration rate (Winter, 1969; Newell, 1980) and in this way may be able to sustain energy flow into growth and reproduction despite increased metabolic energy expenditure at high temperatures (for reviews, see Newell, 1979, 1980; Newell & Branch, 1980). A third group of bivalves, however, shows a uniform rate of filtration over the normal range of thermal tolerance despite the fact that the metabolic rate increases with tempera-

[1] Visiting scientist supported by a Royal Society Senior Research Fellowship.

[2] To whom requests for reprints should be sent.

ture. Griffiths (1980), for example, has found that the filtration rate of specimens of the mussel *Choromytilus meridionalis* from False Bay, South Africa, of 40 mm shell length remained at $\approx 2.8\ l \cdot h^{-1}$ whereas the corresponding oxygen consumption increases from 95 to 370 $\mu l \cdot h^{-1}$ over the range 12–25 °C. It seems likely that in this case either a metabolic energy deficit is incurred where energy expenditure exceeds that gained by filtration at high environmental temperatures, or that changes in absorption efficiency of the ingested ration of this bivalve are alone sufficient to offset increased metabolic losses as the temperature rises.

We have recently shown that preparations of crystalline styles from specimens of *C. meridionalis* which had been acclimated to temperatures between 8 and 22 °C show increased α-amylase activity, a feature which may form an integral part of the improved absorption which is necessary to offset increased metabolic losses during the warm conditions of the summer months (Seiderer & Newell, 1979). Whether this increase in digestive activity of the style was achieved by an increase in enzyme concentration in the style, by an increase in enzyme specific activity or by the occurrence of enzyme variants or isoenzymes of α-amylase was, however, unknown.

The present paper reports the results of studies on the specific activity of α-amylase fractions isolated by anion exchange chromatography from the styles of mussels acclimated to 8 and 22 °C. The data suggest that compensatory adjustments in α-amylase activity following thermal acclimation may indeed reflect the presence of isoenzymes whose occurrence and specific activity is controlled by the temperature to which the mussels have been acclimated.

Materials and Methods

Preparation of material

Specimens of the black mussel *C. meridionalis* (Krauss) were collected from the shore at Blaubergstrand on the west coast of the Cape Peninsula, South Africa, and were placed in shallow trays of aerated sea water some held at 8 °C and others at 22 °C, respectively for a period of 18 days. The mussels were fed once a day with a mixed culture of *Platymonas suecica* Kylin (= *Tetraselmis suecica* (Kylin) Butcher), *Isochrysis galbana* Parke, and *Hymenomonas carteri* (Braarud & Fagerl.) Braarud at a concentration similar to that used by Seiderer & Newell (1979). On the 18th day the crystalline styles of ≈30 mussels from each acclimation regime were removed, rinsed in distilled water and homogenized with 10 strokes of a tight-fitting pestle in a glass homogenizer in 20 ml phosphate buffer pH 9.0 (20 mM Sorensen's phosphate buffer pH 9.0 containing 6.7 mM NaCl). A pH of 9.0 rather than the pH optimum of 7.0 for style α-amylase activity was chosen since this created charge differences between the protein fractions and thus facilitated separation on the column. The homogenate was then centrifuged at 1600 × *g* for 5 min to remove debris and the

supernatant was used immediately for fractionation on diethylaminoethyl cellulose anion exchange resin (Whatman DE 52).

ANION EXCHANGE CHROMATOGRAPHY

The anion exchange resin was de-aerated in a Buchner flask for ≈15 min until no air bubbles were visible and the slurry packed into a 10 × 2.5 cm column. This was then washed with three volumes of phosphate buffer pH 9.0 and the eluate monitored at 280 nm using an ISCO Model UA-5 Absorbance Monitor equipped with a fraction collector.

Approximately 30 A_{280} units of homogenate at a concentration of 10 A_{280} units · ml^{-1} were then applied to the column. The unbound material (i.e., protein not binding to the resin) was removed by flushing the column with phosphate buffer pH 9.0 until the absorbance was minimal. The protein bound to the column was then eluted with a linear gradient of 10–500 mM NaCl in 20 mM phosphate buffer pH 9.0 obtained when 200 ml of 10 mM NaCl and 200 ml of 500 mM NaCl solutions were placed in each of their respective chambers. Fractions of 2 ml were collected throughout elution and used for estimation of α-amylase activity.

ESTIMATION OF α-AMYLASE ACTIVITY

The fractions were then pooled into four groups according to the occurrence of peaks in protein absorbance which corresponds with material which was not bound to the resin, and with three A_{280} peaks which were successively removed from the resin at concentrations of NaCl between 150 and 400 mM. The pooled fractions were then concentrated from ≈50 ml to 5 ml by Amicon filtration and dialysed at 4 °C for 18 h against 500 volumes of 20 mM phosphate buffer pH 7.0 containing 6.7 mM NaCl. The dialysis buffer was changed after 6 and 12 h to ensure that the excess NaCl was removed and to restore the pH optimum of 7.0 for α-amylase activity (Mathers, 1974; Langton, 1977). The concentrated samples were then assayed at pH 7.0 for α-amylase activity using glycogen as substrate and 2,5-dinitrosalicylate to measure the liberated glucose (Bernfeld, 1955; Seiderer & Newell, 1979).

RESULTS

FRACTIONATION BY ANION EXCHANGE CHROMATOGRAPHY

Fig. 1 shows both the absorbance profile at 280 nm of the eluate of crystalline styles from mussels which had been acclimated to 8 °C and the concentration of the NaCl gradient plotted as a function of elution volume. The first protein fraction (Peak I) represents material which remained unbound to the anion exchange resin. A second fraction (Peak II) was eluted from the column at a NaCl concentration of

between 100–150 mM whilst a third protein fraction (Peak III) was eluted in the gradient between 200–250 mM NaCl. Finally, a fourth fraction (Peak IV) was eluted in the NaCl gradient between 300–400 mM.

Fig. 2 shows the results for preparations of crystalline styles which had been removed from mussels acclimated to 22 °C. It is apparent that the first three peaks

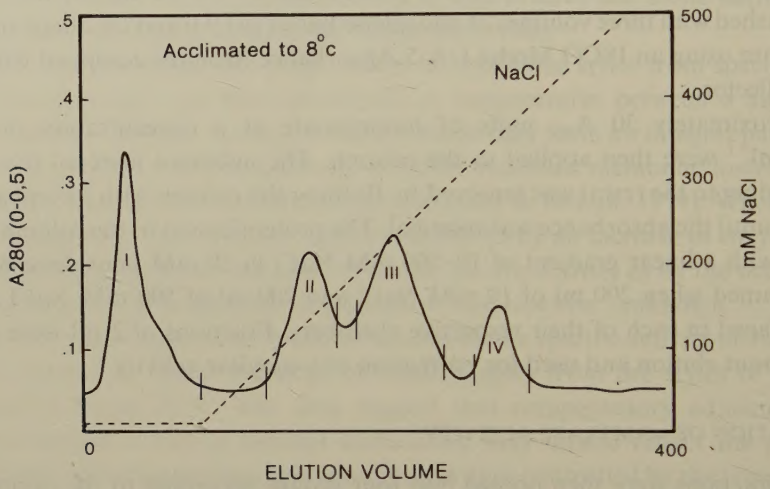

Fig. 1. The absorbance profile at 280 nm of the eluate of crystalline styles from mussels which had been cold-acclimated to 8 °C: the concentration of the NaCl gradient is also plotted as a function of elution volume.

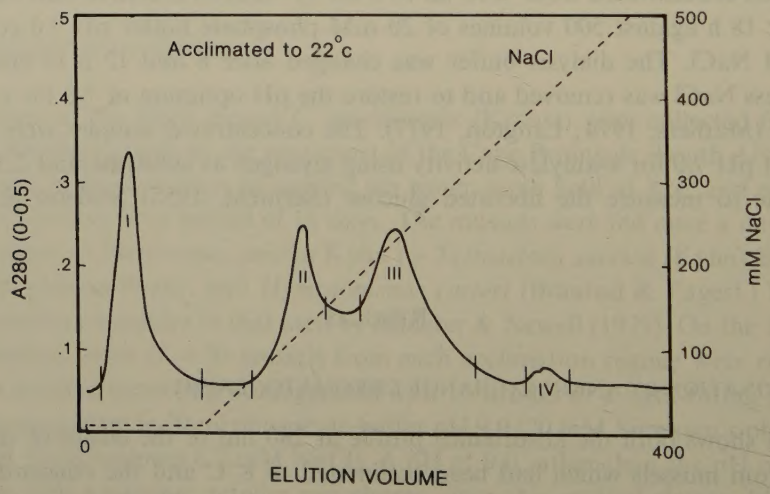

Fig. 2. The absorbance profile at 280 nm of the eluate of crystalline styles from mussels which had been warm-acclimated to 22 °C: the concentration of the NaCl gradient is also plotted as a function of elution volume.

(I–III) correspond generally with those obtained in preparations of styles from cold-acclimated mussels, but that peak IV is much reduced.

The results show, therefore, that the styles from cold-acclimated mussels are characterized by the occurrence of a protein fraction which elutes at 300–400 mM NaCl. Whether differences also exist between component proteins comprising Peak I from cold- and warm-acclimated mussels is not yet known since we have not yet subjected the unbound eluate to further fractionation. The protein comprising Peak IV, however, is unstable and following freezing of the homogenate fails to appear in subsequent elution of the anion exchange column.

α-AMYLASE ACTIVITY IN PROTEIN FRACTIONS FROM THE CRYSTALLINE STYLE

The 2 ml fractions collected following elution of the anion exchange column were pooled into four groups corresponding with the positions of protein Peaks I–IV as shown in Figs. 1 and 2 and concentrated as described on p. 3. The α-amylase activity at pH 7.0 was then measured at 8 and 22 °C.

TABLE I

The specific α-amylase activity assayed at 8 and 22 °C of duplicate samples of protein fractions separated by anion exchange chromatography from the crystalline styles of specimens of *Choromytilus meridionalis* which had been acclimated to 8 and 22 °C, respectively: units expressed as mg glucose liberated from starch substrate per A_{280} unit of protein per 8 min incubation.

Protein fraction	Elution range mM NaCl	Acclimated to 8 °C		Acclimated to 22 °C	
		Assay temperature		Assay temperature	
		8 °C	22 °C	8 °C	22 °C
I	Unbound	0.18	0.18	1.42	4.10
		0.19	0.18	1.42	4.25
II	100–150	0.04	0.05	0.63	2.14
		0.05	0.04	0.66	2.04
III	200–250	0.02	0.02	0.00	0.00
		0.02	0.02	0.00	0.00
IV	300–400	0.47	0.14	0.00	0.00
		0.47	0.13	0.00	0.00
Total specific activity of fractions (mg glucose per A_{280} unit of protein)		0.72	0.38	2.06	6.26

The results are summarized in Table I which shows the specific activity of the enzyme (mg glucose liberated per A_{280} unit of protein per 8 min) obtained on replicate samples for each of the protein fractions assayed at incubation temperatures of 8 and 33 °C. The most obvious feature of the results is that all the protein peaks obtained from the styles of mussels acclimated to a low temperature of 8 °C have some

α-amylase activity but that this is greatest in the peak IV fraction. Activity in this fraction is approximately five times that at an assay temperature of 22 °C and 10–20 times that of the proteins in Fractions II and III. (It is possible that protein Fraction III is a non-amylase fraction and that the small amount of activity found only at 8 °C may represent contamination carried over from Fraction II.) Activity in Fraction I was less than half that in Fraction IV at an assay temperature of 8 °C. Acclimation to 8 °C is thus associated with the occurrence of a protein fraction (Peak IV) with a specific α-amylase activity which exceeds the sum of the activities of all other fractions (Peaks I–III) at an assay temperature of 8 °C.

In contrast, specific activity is very high in protein Fraction I extracted from the styles of mussels acclimated to 22 °C and assayed at 22 °C. It is almost 10 times that of the maximum activity of the most active protein fraction extracted from the styles of mussels acclimated to 8 °C and is reduced with a Q_{10} of ≈ 2 at an assay temperature of 8 °C. The α-amylase in protein Fraction II is also highly active at an assay temperature of 22 °C following acclimation of the mussels to 22 °C and its specific activity is also reduced at the low assay temperature of 8 °C. Finally, no α-amylase activity could be detected in the proteins from Fraction III or the small amount of Fraction IV extracted from the styles of mussels warm-acclimated to 22 °C. Warm-acclimation to 22 °C is thus associated with a major increase in specific α-amylase activity in the second (Peak II) and above all in the first (Peak I) style protein fractions and with a corresponding suppression of activity in Fractions III and IV. Whether the specific activity of proteins from Fractions II and III dominates the α-amylase activity spectrum at intermediate acclimation temperatures between 8 and 22 °C is, however, at present unknown.

Conclusion

Separation of the proteins comprising the crystalline style of the mussel *Choromytilus meridionalis* by anion exchange chromatography shows that three fractions displaying α-amylase activity (Peaks I–III) occur in both warm- and cold-acclimated mussels, but that cold-acclimation to 8 °C results in the appearance of an additional α-amylase fraction (Peak IV) with a specific activity of 0.47 mg glucose per A_{280} unit of protein per 8 min at 8 °C. Following freezing of the style extract, or warm-acclimation to 22 °C for 18 days, there is a reduction of Fraction IV although whether this is due to the presence of proteolytic enzymes in this fraction which destroy the amylase as the temperature is raised is at present unknown. Warm-acclimation also results in a suppression of specific α-amylase activity in Fraction III and a corresponding increase in the activity of Fractions I and II which achieve their maximal α-amylase activity at high incubation temperatures.

The results presented above thus suggest that the functional α-amylase activity of the crystalline style of *C. meridionalis* may be enhanced by the appearance of an

α-amylase fraction (Peak IV) following cold-acclimation which has its maximal activity at low temperatures, and by changes in the activities of existing α-amylase fractions (I–III). Digestive activity is thus increased from a total specific rate of 0.72 mg glucose liberated per A_{280} unit of protein per 8 min incubation at 8 °C to as much as 6.26 following acclimation to 22 °C (Table I).

The occurrence of regulatory isoenzymes which may control the metabolic energy expenditure of ectothermic organisms at a relatively uniform rate despite an increase of temperature is now well-established (see Somero, 1969; Hochachka & Somero, 1972; Markel, 1976; Somero & Hochachka, 1976; Yancey & Somero, 1978) and results in a suppression of metabolic costs in organisms which experience seasonal or latitudinal increases in environmental temperature (for reviews, see Newell, 1979, 1980). The occurrence of digestive isoenzymes whose functional activity may be dramatically enhanced and thus increase components of energy gain in an ectothermic organism following warm-acclimation has, as far as we are aware, not been previously reported. An increase in digestive activity may, however, be of equal importance to a suppression of metabolic costs in the maintenance of energy flow into growth and reproduction in the face of an increase in environmental temperature, especially in organisms such as *C. meridionalis* which do not show a compensatory increase in filtration rate.

Acknowledgements

This work was partly supported by a grant from the South African National Committee for Oceanographic Research to Professor G. M. Branch for research on intertidal organisms and to Professor J. G. Field for the kelp-bed project. It was completed during tenure by R. C. Newell of a Royal Society Senior Research Fellowship.

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J. exp. mar. Biol. Ecol., 1980, Vol. 47, pp. 9–24
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COMPARATIVE FEEDING ECOLOGY OF TWO HERBIVOROUS DAMSELFISHES (POMACENTRIDAE: TELEOSTEI) FROM THE GULF OF CALIFORNIA, MEXICO

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Abstract: Two species of benthic damselfishes from the Gulf of California, Mexico, use contrasting behaviors when feeding on benthic algal communities. The small (±70 g) Cortez damselfish, *Eupomacentrus rectifraenum* (Gill, 1862), feeds selectively from a multi-species algal mat, eats fleshy red and green algae and ignores brown and calcareous algae. The giant blue damselfish, *Microspathodon dorsalis* (Gill, 1862), is a large (±450 g), lethargic, nonselective feeder which grazes on a near monoculture of a fleshy red alga, *Polysiphonia* sp. Feeding activity for both species is low in the morning, peaks during late afternoon, and drops sharply as night approaches. Based on feeding rates, gut-filling times, and weights of gut contents, Cortez damselfish process six to eight full guts of food and giant blue damselfish three full guts of food per day. The algal mat exhibits high standing crops (291–618 g dry wt $\cdot m^{-2}$) and low productivity, but the preferred food of the Cortez damselfish (*Ulva*) appears to colonize the mat frequently and grow rapidly. The *Polysiphonia* dominated community on giant blue damselfish territories exhibits low standing crops (23 g $\cdot m^{-2}$) and high productivity (34–47 times that of the mat per gram algae). Even though the feeding behaviors and resources used by the two damselfishes differ, both species eat similar foods (delicate red and green fleshy algae) and depend on rapid colonization and/or high productivity to maintain their primary foods in the grazed algal community.

INTRODUCTION

Herbivorous fishes are regarded as important regulators of algal community structure and productivity on tropical reefs (Brawley & Adey, 1977; Kaufman, 1977; Ogden & Lobel, 1978; Montgomery, 1980), but the ecological processes by which this regulation is brought about are not entirely clear. Studies of gut contents and feeding behavior clearly demonstrate herbivorous feeding in many groups of marine teleosts (blennies, damselfishes, parrotfishes, rudderfishes, surgeonfishes, rabbitfishes, etc.) (Hiatt & Strasburg, 1960; Randall, 1967; Hobson, 1968; Tsuda & Bryan, 1973; Montgomery, 1977). Estimates of fish populations made with SCUBA and snorkeling techniques reveal tropical reef fish standing crops of 50–190 g $\cdot m^{-2}$ (Brock, 1954; Bardach, 1959; Randall, 1963), of which 15–25% comprise herbivorous and omnivorous species (Ogden & Lobel, 1978). This research provides a rich background from which my studies on food availability, food selection, grazing rates, and algal productivity were designed.

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Two herbivorous damselfishes (Pomacentridae) on a boulder reef in the lower Gulf of California, Mexico are closely related; both feed on fleshy red or green algae, but their feeding behavior and ecology contrasts sharply. The smaller Cortez damselfish (*Eupomacentrus rectifraenum* (Gill, 1862)) is a selective feeder, while the larger giant blue damselfish (*Microspathodon dorsalis* (Gill, 1862)) is nonselective. This research was directed at the relative abundance and availability of various algae to the two damselfishes. This led to estimates of abundance and territory size of the two species and measures of their grazing rates through studies of biting and gut-filling rates. Grazing rates are intense on preferred foods of both fishes, but the impact of grazing on the total algal community depends heavily on the degree of selectivity each fish exhibits. Frequent colonization and high productivity appear to underlie persistence of preferred foods in algal communities grazed by the Cortez and giant blue damselfishes.

Materials and Methods

SITE

The study site was a granite boulder reef along the south face of Los Frailes Mountain, Baja California Sur, Mexico (23°23′N; 109°25′W; Montgomery, 1980) during May–June 1976, July–August 1977, November 1977, and January 1978. Collections and observations were made at depths of 3–5 m along the upper edge of a steep slope of large boulders. The most common damselfish on these reefs is the Cortez damselfish, *Eupomacentrus rectifraenum*, a small ($\leq$120 mm standard length) fish which defends territories around the bases of boulders. The giant blue damselfish, *Microspathodon dorsalis* ($\leq$220 mm standard length), defends year-round territories near the tops of large boulders rising above the surrounding boulder-cobble substratum. A 0.5–1 cm thick algal mat composed of many species of red, green, and brown algae blankets most rock surfaces. A near monoculture of a filamentous red alga, *Polysiphonia* sp. (Ceramiales; Hollenberg, 1961), covers giant blue damselfish territories.

SAMPLING METHODS

Algae

Samples of the algal mat were taken from areas where Cortez damselfish were collected. Samples of algae in giant blue damselfish territories were removed prior to collection of the resident fish. Standing crops of the algal mat were determined by scraping algae from known areas (12.9 cm^2) on the surfaces of granite cobble after cobbles had been returned to the beach. Because fragments of boulders bearing giant blue damselfish territories could not be removed intact or the boulders scraped in situ, standing crops of *Polysiphonia* were estimated by scraping known areas of latex

rubber tubing used to anchor experimental enclosures (see Montgomery, 1980) over territories. The tubing was rapidly colonized by *Polysiphonia*; standing crops on sampled tubing appeared similar to that on territory surfaces. Samples for standing crop estimation were air-dried in the field and returned to the laboratory for drying to constant weight at 70 °C. Samples for identification and counting of algae were preserved in 5% formalin. During the remainder of the paper, algal mat will refer to Cortez damselfish feeding areas and *Polysiphonia* to giant blue damselfish feeding territories.

Biting rates

I counted biting rates of Cortez and giant blue damselfish while snorkeling at the surface 3–4 m from the fish. Time was measured from immediately after a bite (Bite 0) to immediately after Bite 5. This interval, determined to the nearest 5 s, included five bites and five inter-bite intervals. The five-bite interval provided a better record of average feeding activity of an individual than a one- or two-bite interval and allowed survey of a large number of fish per hour. Only bites accompanied by strong pectoral and caudal fin action were recorded immediately. Questionable bites were counted if they were followed within 5 s by vigorous chewing motions or expulsion of material from mouth or operculum.

Territory size

Giant blue damselfish defend well-defined territories on boulder surfaces. These territories were measured by comparison to a 0.1-m^2 frame placed on the territory. Boulder surfaces clearly dominated by *Polysiphonia* were considered to be within territory boundaries.

In contrast, Cortez damselfish occupy home ranges, portions of which may be foraged by more than one fish. The center of these home range activities is a shelter hole in the boulder substratum, and the fish rarely move more than 1 m from this focus. Occasional defensive attacks are directed against neighboring small fishes that are potential competitors for food or space (conspecifics, other *Eupomacentrus* spp., or a common blenny, *Ophioblennius steindachneri*). Vigorous defensive attacks are, however, directed at larger herbivorous fishes (parrotfishes, surgeonfishes, rudderfishes) whenever they attempt to invade the feeding area. Because the area over which a Cortez damselfish forages exclusively is ill defined, the average size of the feeding area was estimated from the inverse of population density (fish $\cdot$ m^{-2}) as the area of substratum (m^2) per individual fish.

Population estimates

Densities were measured in the same areas that samples of fish and algae were collected. Eight master quadrats (each 25 × 2 m) were laid out with nylon rope

in non-overlapping areas. Each master quadrat was then subdivided into five smaller quadrats of 5 m length each (total 40 quadrats). I scored the number of adult damselfish within each 10-m^2 quadrat during a 15-min survey of the master quadrat. Population estimates were made by this method on 4 days during January 1978. Densities did not change noticeably during the course of the study, however, and the mean density of the Cortez damselfish in January 1978 (0.51 fish $\cdot$ m^{-2}) is in accord with densities obtained at the same site with a similar method during November 1972 (0.55 fish $\cdot$ m^{-2}, $N = 11$).

Gut contents

Fish intended for gut content studies were speared in areas adjacent to those where behavioral studies were conducted. For gut filling studies, specimens ($\bar{X} = 3$, range 1–6) were collected in the morning at approximately 1-h intervals. Specimens intended for gut content studies were collected in early afternoon, when feeding was most intense and rarely interrupted by social or other behaviors. Dissections were completed within 1–2 h of capture or specimens were preserved in 10% formalin for return to the laboratory. Methods for measuring gut morphology follow those of Montgomery (1977). The alimentary canal was removed by cutting the esophagus and the termination of the rectum. The intestine was measured without stretching after separation from the stomach immediately posterior to the pyloric caecae. Distance from pylorus to the leading bolus of food was also measured to allow calculation of the percent intestine filled with food.

Gut volume

Gut content volumes were measured by placing material in a cuvette graduated in 0.1-ml divisions. Sufficient 5% formalin was added from a burette graduated in 0.05-ml divisions to bring the total volume to 1.0 or 1.5 ml. Sample volume was estimated to the nearest 0.05 ml by subtracting fluid volume added from total volume.

SPECIES COMPOSITION OF ALGAE AND STOMACH CONTENTS

A modified form of Jones' (1968) point-intersection technique to assess species composition of algae and stomach samples was used (Montgomery, 1978, 1980). Samples were spread over the bottom of a large sorting Petri dish scored by a grid of 2-cm squares. An ocular grid in a 10 × stereomicroscope was centered over each square to be counted. Material beneath each of 81 intersections of horizontal and vertical grid lines was identified and scored. Sand grains, shell fragments, and no material were scored as zero, while biological material that could not be assigned to a particular taxon was scored as unidentified. Eight squares were counted per sample.

Sample sizes are small ($N = 6$) for fish used in comparisons of stomach contents

with available foods (Tables I, II). Results presented here derive from a broader study of phenomena associated with successful herbivory in marine shore fishes, portions of which required additional collections (Montgomery, 1980; Montgomery & Gerking, 1980). Although larger or seasonal samples would have better defined the presence or absence of selectivity by these two fishes, the data are sufficient to demonstrate the important trends. In addition, at least for the giant blue damselfish, more extensive collections would have seriously depleted the study population.

OXYGEN PRODUCTION EXPERIMENTS

A variation of the light–dark bottle technique was used to estimate the relative productivity of both algal mat and *Polysiphonia* on boulder surfaces. Light chambers constructed of clear Plexiglas and dark chambers of PVC pipe were tied to rock surfaces with latex rubber tubing stretched taut. Algae crushed below edges of the chambers and a 0.6-cm thick neoprene gasket (January only) improved the seal of chambers against rock surfaces. Experiments were repeated on 4 days in November 1977 and 8 days in January 1978; each daily experiment consisted of three sets of light and dark chambers placed over the algal mat and two sets on *Polysiphonia* dominated territories, giving a final total of 34 replications for the algal mat (only one set was made on 4 November) and 24 replications for *Polysiphonia*. Tests were conducted on clear, sunny days and at depths of 3–4 m. Experiments spanned 2.5–6.5 h periods (between 0930 and 1500), but the time of day and interval were identical for all replicates on a given day.

Water samples were drawn into 35-ml syringes at the beginning (control) and end (experimental) of the test period, immediately fixed with manganous sulphate and alkaline iodide reagents, shielded from direct sunlight, and transported to the beach. Dissolved oxygen was determined by the Winkler method (Strickland & Parsons, 1960, 1972). Standing crops used to calculate weight-specific productivity were determined as described in previous sections.

RESULTS

SIZE OF DAMSELFISH FEEDING AREAS

Average size of the feeding area is estimated from the population density. Density of Cortez damselfish was 0.51 fish $\cdot$ m^{-2} (SE = 0.03, N = 40 quadrats), about 2.0 m^2 per fish, in January 1978. Giant blue damselfish, in contrast, were usually restricted to the upper surfaces of boulders 2–3 m in diameter which extend well above the basal substratum. The average giant blue damselfish territory covers 0.51 m^2 (SE = 0.03, N = 26).

The true feeding area for the Cortez damselfish is overestimated slightly from adult population density. The Cortez damselfish maintains home ranges with two

congeners (*Eupomacentrus flavilatus* and *E. acapulcoensis*) of similar size and ecology which occur at a mean density of 0.09 fish · m^{-2}. The inverse of total density of all three species (0.6 fish · m^{-2}) gives an average feeding area for individual *Eupomacentrus* of 1.7 m^2 per fish. These areas are also sporadically grazed by other herbivores (blennies, rudderfishes, surgeonfishes, and parrotfishes), but their influence on food resources was not estimated. Juvenile damselfish were also excluded from consideration because their food habits were not studied. Emery (1973) found that juveniles of *Microspathodon chrysurus* and four Caribbean species of *Eupomacentrus* eat more animal material than the adults.

STANDING CROPS OF ALGAE

The standing crop of the algal mat decreased significantly ($P < 0.02$ by Mann-Whitney *U*-test; Zar, 1974) from 618 g dry wt · m^{-2} in August 1977 (SE = 58, $N = 21$) to 291 g · m^{-2} in January 1978 (SE = 68, $N = 7$), with coefficients of variation of 31% and 62%, respectively (Montgomery, 1980). The decline suggests either a change in primary productivity from summer to winter or a change in grazing rates, but this investigation did not reveal which of these alternatives is more plausible. The high variability in standing crops is attributed to patchiness of the algal mat. Cobbles were collected from the same depth and were not shaded by surrounding boulders, suggesting that temperature, total insolation, and spectral differences in incident light were not responsible for the variation. The product of feeding area and the standing crop of algae on that feeding area is the biomass available to the Cortez damselfish. This value was estimated at 1236 g dry wt during August 1977 and 582 g during January 1978.

The standing crop of *Polysiphonia* was estimated at 22.8 g · m^{-2} (SE = 2.0, $N = 10$) during August 1977; standing crops do not appear to change throughout the year (Montgomery, 1980). The standing crop on an average giant blue damselfish territory (0.51 m^2) is 11.6 g (Montgomery, 1980).

AVAILABLE AND SELECTED FOODS

Eupomacentrus rectifraenum

The algal mat contains over 30 species of algae. These can be grouped as calcareous and fleshy (filamentous) red, leafy green, filamentous green and blue-green, and leafy brown algae (Table I). Based on point-intersect counts, filamentous red algae make up 32.7%, filamentous green and blue-green algae 25.0%, calcareous red algae 16.5%, leafy brown algae 11.4%, and leafy green algae 0.6% of the total biological material in the mat. Unidentified algae account for 13.4% and animals 1.5% of the total.

Cortez damselfish feed selectively from the algal mat, virtually ignoring calcareous and brown algae and choosing filamentous red algae and a leafy green alga, *Ulva*

(Table I). On the average, calcareous algae represented 6.6% of the diet (4.6% if one excludes an unusually high value of 16% for one fish) but 16.5% of the mat, while brown algae made up 11.4% of the mat but only 1.1% of the stomach contents.

TABLE I

Average composition of the algal mat at Los Frailes compared with stomach contents of Cortez damselfish: samples of fish and algae were collected August 1977; values are percent of total biological material in 11 algal samples and 6 fish stomachs.

	Algal mat		Stomachs*	
	$\bar{X}$	SE	$\bar{X}$	SE
Calcareous red algae				
Jania	13.01	3.07	5.95	1.96
Amphiroa	3.50	0.83	0.61	0.43
Fleshy red algae				
Gracilaria	19.17	3.77	36.42	4.40
Ceramium	7.86	1.29	7.13	3.18
Polysiphonia	2.54	0.75	7.25	2.47
Other	3.13	1.69	2.23	0.99
Filamentous green and blue-green algae	24.95	5.63	8.19	1.94
Leafy green algae				
Ulva	0.61	0.38	16.11	4.11
Leafy brown algae				
Dictyota	8.27	4.94	1.07	0.71
Padina	3.12	3.12	0	....
Animal	1.46	0.34	2.42	1.22
Unidentified	13.39	2.82	12.63	1.44
Total point intersect counts	2270		991	

* Contents of all stomachs differed from the average algal mat composition at 0.01 level by the Smirnov test (Conover, 1971).

The proportion of filamentous red algae in the stomach was 1.6 times that of the algal mat, indicating that filamentous red algae are selected. The most striking selectivity was for the leafy green alga, *Ulva*, which made up 16.1% of stomach contents but only 0.6% of the mat, a 26-fold difference. Some exclusion of filamentous green and blue-green algae from the diet may occur; stomach values were one-third of the algal mat values. Percent animal material in stomachs (2.4%) was 1.6 times that of the algal mat. This low degree of selectivity for a rare food source suggests that animals are eaten as they are encountered, but are certainly preferred over calcareous, brown, and filamentous green and blue-green algae.

Detailed conclusions based on so few specimens ($N = 6$) need be viewed with caution, but the important generalities are sound: Cortez damselfish are selective feeders which choose *Ulva* (fleshy red algae to a lesser extent) and shun brown algae (and

probably calcareous forms as well). These findings are consistent with general surveys of gut contents of 37 specimens in November 1972 (unpubl.) and are bolstered by observations of feeding behavior. Cortez damselfish were never seen biting species of brown algae (*Dictyota*, *Padina*, *Sargassum*), but often eat filamentous red algae growing at the bases of the large brown algae. Further, the fish spend a great deal of their foraging time inspecting the algal mat from a distance of 5 cm or less, as if to select particular foods before they bite.

Microspathodon dorsalis

Giant blue damselfish, in contrast to Cortez damselfish, are nonselective feeders (Table II), eating the algae on their territories in nearly the same proportions in which they occur (Montgomery, 1980). On the average, territories comprised 79.4% fleshy red algae (stomach 77.3%), 9.4% filamentous green and blue-green algae (stomach 5.8%), 5.0% *Ulva* (stomach 0.2%), 0.5% calcareous red algae (stomach 1.6%), and no brown algae (stomach 0.3%). The average for *Ulva* was inflated by a 21.5% value from one territory. *Polysiphonia* constituted 59.4% of material on territories and

TABLE II

Average composition of stomach contents from giant blue damselfish compared with algae on their territories: values are percent of total biological material on territories and in stomachs of six giant blue damselfish collected August 1977.

	Territories*		Stomachs*	
	$\bar{X}$	SE	$\bar{X}$	SE
Calcareous red algae				
Jania	0.45	0.29	0.66	0.26
Amphiroa	0.07	0.07	0.92	0.65
Fleshy red algae				
Gracilaria	4.28	1.66	8.19	3.22
Ceramium	10.88	2.93	5.76	2.52
Polysiphonia	59.38	8.29	60.49	9.67
Other	4.85	2.25	2.84	1.38
Filamentous green and blue-green algae	9.42	2.63	5.77	2.13
Leafy green algae				
Ulva	5.03	4.06	0.20	0.20
Leafy brown algae				
Dicyota	0	–	0	–
Padina	0	–	0.27	0.27
Animal	0.20	0.14	0.79	0.44
Unidentified	5.43	1.43	14.13	3.56
Total point intersect counts	1956		1142	

* P(territories = stomachs) ≥ 0.20 by the Smirnov test (Conover, 1971). $P \geq 0.20$ for five of six individual comparisons as well; the sixth comparison gave $P > 0.1$ (Montgomery, 1980).

60.5% of stomach contents. The difference between percent animal material in stomachs (0.8%) and on territories (0.2%) is probably due to sampling error rather than selectivity. Animal materials in the gut were either small (<2-3 mm) and cryptic crustaceans, fragments of these crustaceans, or minute (1–2 mm) snails distinguishable from sand particles only upon scrutiny.

As for the Cortez damselfish, conclusions based on the specimens detailed here are supported by observations of feeding behavior. Giant blue damselfish usually move to a portion of the territory, pitch forward, and bite vigorously without apparent inspection of the substratum. In contrast to the Cortez damselfish, the mouth of a giant blue damselfish is much larger than the 1–2 cm long, delicate filaments on which it feeds, and a bite clears most algae from a circular patch of substratum 3–4 cm in diameter. There is no indication of selective feeding other than the choice of a general zone within the territory from which a bite is taken.

GRAZING RATES

Eupomacentrus rectifraenum

The Cortez damselfish had the same daily pattern of biting in all seasons. Biting rates were low in early morning, rose to a maximum in late afternoon, and dropped rapidly toward dark (Fig. 1). Feeding day length extended approximately from beginning to end of civil twilight (time beginning before sunrise or ending after sunset when the sun is about 6° below the horizon; U.S. Naval Observatory, 1974, 1975,

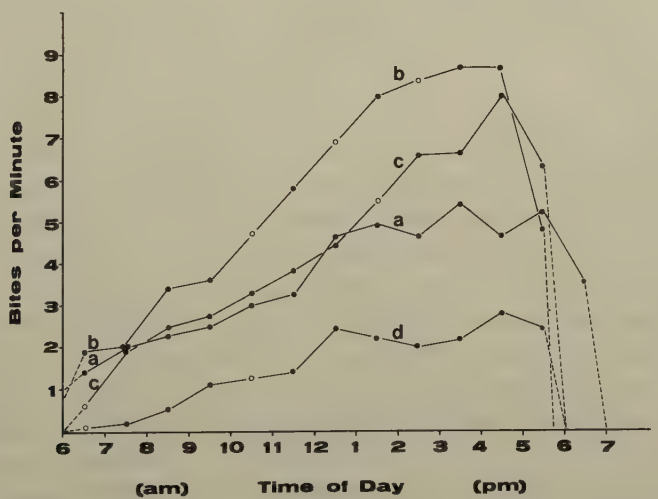

Fig. 1. Biting rates of Cortez and giant blue damselfishes at Los Frailes: ●, averages of the biting rates of individual fish observed during a 1-h period; ○, estimates via linear extrapolation from flanking values; the total number of Cortez damselfish observed was 405 (individuals per data point: range 6–45, mean 31) during June 1976, (a) 138 (6–29, 15) in November 1977 (b) and 159 (6–25, 16) in January 1978 (c); 88 (6–15, 10) giant blue damselfish were observed in January 1978 (d).

1976). These periods coincided with times when fish became active in the morning and inactive in the evening and were the earliest and latest times that reliable observations were possible underwater. The estimated number of bites taken by a Cortez damselfish during a feeding day is calculated from hourly biting rates and feeding day length (Table III). The number of bites per day in November 1977 (3996) exceeded estimates for June 1976 (2891) and January 1978 (3188).

TABLE III

Day lengths and water temperatures at Los Frailes during periods when feeding rates were estimated for damselfishes: estimated number of bites taken per day by Cortez damselfish (giant blue damselfish in parentheses) is included.

Dates	1–6 June 1976	4–8 November 1977	4–7 January 1978
Begin civil twilight	0455	0541	0612
End civil twilight	1901	1746	1758
Feeding day length (h)	14.10	12.10	11.77
Water temperature (°C)	27	29	23
Total bites per day	2891	3996	3138 (1106)

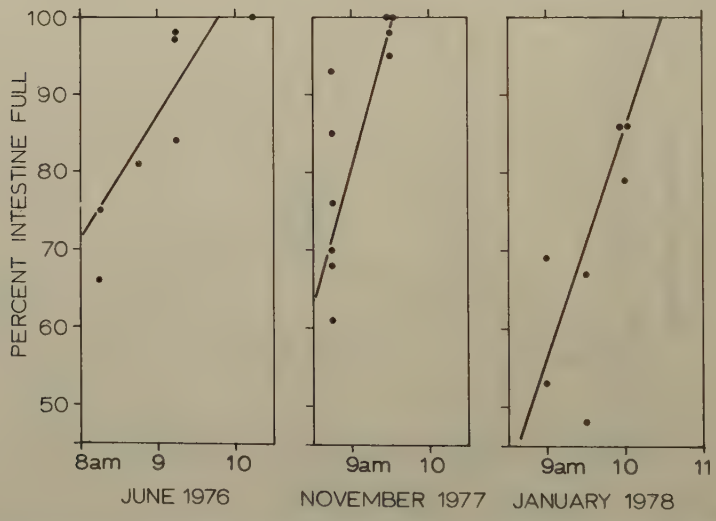

Fig. 2. Intestinal filling in Cortez damselfish: ●, measurements from dissected fish; lines are regressions of percent intestine full against time; intersections of regression lines with 100% were taken as the average times of gut filling for subsequent calculations; the regression equations and correlation coefficients were: for June 1976, $y = 16.15x + 20.70$, $r = 0.87$; for November 1977, $y = 32.50x - 25.25$, $r = 0.80$; for January 1978, $y = 29.31x - 24.82$, $r = 0.72$.

The earliest time of day at which the gut was 100% full was calculated from a linear regression of percent filled against time of day, using points from sunrise to the first time interval when a specimen had a full intestine (Fig. 2). The estimate for the time at which the gut was first filled in June was 0951, in November 0932, and in January 1028. These times corresponded to 4.91, 3.85, and 4.26 h after the beginning of civil twilight, respectively. On the average, a full gut contained 2.14 ml (SE = 0.9, $N = 9$) of food, wet volume. This does not take into account reduction in volume due to absorption. The algal mat from which Cortez damselfish fed weighed 0.69 g dry wt $\cdot$ ml^{-1} wet volume (SE = 0.07, $N = 15$), so that a filled gut weighed about 1.48 g dry wt. This assumes similar densities for all components of the mat. Filled gut weight is probably overestimated because the fish tend to eat plants with relatively low density compared to calcareous and heavy-bodied brown algae. This estimate is for an adult Cortez damselfish of about 100 mm standard length and 70 g total wet wt.

Microspathodon dorsalis

Gut filling rates for January 1978 are based on only five specimens. Two specimens taken at 1230, 6.25 h after the beginning of civil twilight, had intestines 80–85% full, while three specimens collected 2 h later were 100% full. No information was obtained at other times of the year. Hourly biting rates for January 1978 indicate that 338 bites would be taken before 1230. Extrapolating linearly from this value to 100% full gives estimates of 397–423 bites required to fill the gut. On the average, there are 2.72 g dry wt (SE = 0.51, $N = 10$) of algae in a full stomach and intestine of an adult giant blue damselfish of 200–220 mm standard length and about 450 g body wt.

PRODUCTIVITY OF ALGAL COMMUNITIES

On a per unit area basis, relative productivity of *Polysiphonia* within giant blue damselfish territories averaged 2.5 times that of the algal mat during both November 1977 and January 1978, but the *Polysiphonia* community was 34–47 times as productive as the algal mat on a per gram basis (Table IV). Weather and water were clear and water temperatures constant (29 °C in November, 23 °C in January) during each series of tests. The oxygen production estimates are probably too low to allow accurate calculation of carbon fixation. The November light–dark bottle attempts were subject to undetermined amounts of leakage because of poor sealing against the rough boulder surfaces. In January a neoprene gasket glued to the lip of each box reduced leakage.

The large variances for results were probably not due to differences in depth, weather, location of light vs. dark boxes, or clarity of water. All experiments were conducted at the same depth (3 ± 0.5 m), weather was clear and sunny on all days except one, light and dark chambers were placed on the same rock face within 10–30 cm of each other, and underwater visibility on test days was at least 10–15 m. Dif-

ferences between time or length of runs on different days probably contributed to large variances, but these parameters did not differ between mat and territories on a given day. In addition, local variation in mat composition and disturbance of chambers by pugnacious damselfish probably contributed to wide ranges in values. One algal mat site yielded production values 2–3 times those of other sites; subsequent scrutiny revealed an unusual preponderance of delicate, fleshy red algae. On several occasions, giant blue damselfish rammed experiments set on their territories, and one fish dislodged the entire apparatus by butting chambers repeatedly.

TABLE IV

Oxygen production by algal communities: giant blue damselfish territories compared with surrounding algal mat; data expressed per m^2 surface area and per gram standing crop*.

	mg $O_2 \cdot m^{-2} \cdot h^{-1}$		mg $O_2 \cdot m^{-2} \cdot h^{-1} \cdot g^{-1}$	
	Algal mat	Territories	Algal mat	Territories
4–7 November 1977				
Mean	8.4	21.4	0.02	0.94
SE	2.9	5.5	0.01	0.24
N	10	6	10	6
3–16 January 1978				
Mean	18.7	45.9	0.06	2.01
SE	2.6	9.8	0.01	0.43
N	23	14	23	14

* Standing crops used in calculations: algal mat, 1977 = 422 $g \cdot m^{-2}$; 1978 = 391 $g \cdot m^{-2}$; territories, 1977 and 1978 = 22.8 $g \cdot m^{-2}$.

In spite of such variation, production by *Polysiphonia* clearly exceeded that of the algal mat. Algal mat and territory production differ significantly ($P < 0.05$) by both the Smirnov and Mann–Whitney *U*-tests (Tate & Clelland, 1957; Conover, 1971). Daily comparisons demonstrate that both daily maximum and minimum values for *Polysiphonia* exceeded the respective maxima and minima for the mat on 10 of 12 test days, and *Polysiphonia* means were greater than algal mat means on 11 of 12 days. Therefore, these estimates of photosynthetic activity are considered valid when viewed relative to other tests during the same period rather than as accurate measures of true primary productivity.

DISCUSSION

FOOD OF DAMSELFISHES

The almost continuous feeding of the Cortez damselfish contrasts sharply with the infrequent biting of the giant blue damselfish, but both types of behavior produce similar results: fleshy red and green algae are ingested, while brown and calcareous

algae are not. These results are common to other reef fishes as well. Tropical rabbitfishes (Siganidae; Tsuda & Bryan, 1973; Von Westernhagen, 1974), damselfishes (Dennis Lassuy, pers. comm.), and temperate blennioids (Montgomery, 1977) also eat fleshy red and green algae and ignore brown and calcareous algae.

A Cortez damselfish has 582–1236 g dry wt of algal mat within its 2-m^2 feeding area, about 34% of which is preferred filamentous red and leafy green algal foods. The 11.6 g of available algal standing crop on a giant blue damselfish territory is almost exclusively *Polysiphonia* sp. If weight of edible red or green algae is related to weight of fish, an adult Cortez damselfish (70 g) had 2.8 to 6.0 g edible algae per gram of fish, compared to the giant blue damselfish (450 g) which has only 0.03 g algae available per gram of fish on its 0.51-m^2 territory.

CORTEZ DAMSELFISH GRAZING RATES

Most grazing fishes feed regularly throughout the day and are inactive after dark (Hobson, 1974; Smith & Paulson, 1974). The Cortez damselfish initiates active feeding shortly before sunrise and continues until dark. The average interval between bites was 10.9 sec at the maximum daily biting rate recorded for this fish (3996 bites · day^{-1}). At the lowest daily biting rate (2891 bites · day^{-1}) the average interval increased to 17.6 sec. Feeding, therefore, takes up a major portion of time during which the fish is active, as the inter-bite intervals are largely involved with searching for and handling (culling and chewing) food.

From the mean hourly biting rates and time required to fill the gut, the average number of bites required to fill the gut once was 501 in June 1976, 535 in November 1977, and 549 in January 1978. The number of complete fillings of the gut during the day (calculated by dividing total bites per day by bites required to fill the gut) was 5.8 in June, 7.5 in November and 5.7 in January. Based on average volume of a full gut (2.14 ml) and weight of the algal mat (0.69 g dry wt · ml^{-1}), a Cortez damselfish probably ingested less than 8–11 g dry wt of algae per day.

GIANT BLUE DAMSELFISH GRAZING RATES

Calculations of food intake for the giant blue damselfish are based solely on data from January 1978. The diel cycle of feeding activity is similar to that of the Cortez damselfish, but the giant blue damselfish feeds much less frequently than the smaller fish. On the average, 38.4 sec separate bites of the giant blue damselfish compared with 10.9 sec for the Cortez damselfish during the same season. The near monoculture of *Polysiphonia* within the territories reduces search time for food to almost zero.

An adult giant blue damselfish takes 1106 bites per day with 397 bites needed to fill the gut. The fish, then, processes 2.8 full guts of food per day amounting to about 7.6 g of algae.

Results from Cortez and giant blue damselfishes illustrate three main points.

First, both species exhibit the same diel cycle of feeding behavior. At least for the Cortez damselfish, this cycle may change quantitatively, but not qualitatively, from season to season. Secondly, estimates of daily food intake based only on gut evacuation rates and day length (Bardach, 1961) must be re-evaluated, because cyclic daily changes in biting rates violate the underlying assumption of constant ingestion rates. Bardach (1961) estimated that small fish (10–50 g) process three full intestines of food per day, roughly one-half the amount processed by the Cortez damselfish. The 2.8 intestines of food ingested per day by the giant blue damselfish is approximately 1.5 times the amount predicted by Bardach (1961) for 250–500 g fishes. Thirdly, because herbivorous shore fishes may spend a large fraction of their time feeding, the relationship between feeding and allocation of time to other activities deserves further attention.

IMPACT OF GRAZING ON ALGAL RESOURCES

The fraction of the total standing crop in a feeding area that is removed per day by a Cortez damselfish is small. In January, 1978, the maximum daily ration (about 8 g) was 1.4% of the standing crop in the 2.0-m^2 feeding area (582 g). Including the other species of *Eupomacentrus* (*E. acapulcoensis*, *E. flavilatus*) in the estimate raises the fraction of the algal mat removed by damselfishes to only 1.7% per day. On this basis, Cortez damselfish appear to exert little impact on the total algal community.

The impact of grazing on the preferred food of the Cortez damselfish (*Ulva*) is, however, much greater. The maximum standing crop of *Ulva* available on a Cortez damselfish feeding area probably ranges between 3.5 and 7.5 g dry wt, while ingestion amounts to about 1.5 g/day or 20–40% of the biomass. Also, most plants taken from Cortez damselfish stomachs are not fragments bitten from large plants but are entire specimens <1 cm in width, including the holdfast. Cortez damselfish are thus eating young colonists rather than older, established plants. Clearly, the frequency of colonization and the primary productivity of *Ulva* must be high to allow that plant to persist in the face of such grazing pressure.

The giant blue damselfish also strongly influences its primary food, *Polysiphonia*, as well as any other algae that settle on its small territory. When territories are caged to exclude giant blue damselfish, the *Polysiphonia* dominated community is rapidly invaded by several species of algae characteristic of the algal mat (Montgomery, 1980). Giant blue damselfish do not selectively overgraze these algae. Instead, *Polysiphonia* appears to be the only alga with a growth rate sufficient to persist under the influence of intense, nonselective grazing (Montgomery, 1980). The estimated daily ration of 7.1–7.6 g dry wt of algae is 62–67% of the standing crop available to the fish. A giant blue damselfish would eat the entire standing crop of its territory in less than 2 days were it not for high productivity of *Polysiphonia* (Montgomery, 1980).

In summary, Cortez damselfish do not greatly affect the algal mat except through heavy grazing of the preferred food, *Ulva*. In contrast, giant blue damselfish influence

all algae that settle on their territories; persistence of *Polysiphonia* in the face of tremendous grazing pressures clearly depends on very high productivity. *Polysiphonia* was 34–47 times as productive as the total algal mat on a per gram dry wt basis. At first glance this difference in productivity suggests that Cortez and giant blue damselfishes feed on resources which differ greatly in their productivity. Productivity measurements for *Ulva* alone were impossible, but the persistence of the plant in the algal mat appears to depend on frequent recolonization and high growth rates. Thus, the Cortez and giant blue damselfishes, although differing greatly in feeding behavior, both depend on delicately structured red or green algae which exhibit high productivity.

Acknowledgements

Invaluable aid in the field was provided by S. J. Montgomery, D. Rickard and H. Meter. Dr. S. D. Gerking offered critical advice throughout all phases of the study and critically edited the manuscript. Financial support was provided by National Science Foundation Grant OCE 77-09211 to S. D. Gerking and by graduate research funds from the Department of Zoology, Arizona State University. Finally, a sincere note of thanks goes to Bob and ChaCha Van Wormer of Hotel Punta Colorada, Baja California Sur; this work would have been impossible without the generosity, gracious hospitality and logistic support of these friends and their staff.

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J. exp. mar. Biol. Ecol., 1980, Vol. 47, pp. 25–33
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MARINE STEROLS. XII. THE STEROLS OF SOME PELAGIC MARINE CRUSTACEANS

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Abstract: The sterol composition of some pelagic crustaceans (three species of pelagic euphausiids, one species of a bathypelagic marine mysid and one species of an estuarine mysid, two species of pelagic marine copepods, and one species of a bathypelagic marine copepod) have been determined by gas chromatographic mass spectrometric techniques. The euphausiids appear to have developed an almost unispecific requirement for cholesterol, irrespective of trophic level, whilst the mysids show a considerable variation in sterol composition. The two near-surface copepod species contain a significantly greater variety of sterols than their bathypelagic counterpart. The results are compared with the known sterol compositions of other crustaceans in the marine food web and with phytoplankton. The extent of modification of dietary sterols after ingestion is discussed.

INTRODUCTION

Whilst the sterol chemistry of some of the marine and freshwater crustaceans has received considerable attention (see reviews by Goad, 1976, 1978; Morris & Culkin, 1977) comparatively few data are available on the sterol composition of pelagic marine copepods, euphausiids, and mysids. One species of euphausiid (*Nyctiphanes norvegica*) has been analysed by Idler & Wiseman (1971), one species of mysid (*Neomysis intermedia*) by Teshima & Kanazawa (1971), and one species of copepod (*Euchaeta japonica*) by Lee *et al.* (1974).

These crustacean groups are major components of the marine ecosystem. The copepods especially dominate the marine zooplankton in numbers and together with the other small crustaceans provide the link between the primary producers in the marine food web, the phytoplankton, and the larger carnivorous plankton. Knowledge of their sterol chemistry and hence their contribution to the pool of sterols in the marine food web is necessary for a proper understanding of the relative requirements of sterols by marine organisms and the biochemical potential for sterol synthesis-modification within the various trophic levels. Similarly such knowledge is necessary if the geochemical pathways of sterol diagenesis during and after sedimentation of dead organisms are to be confidently identified.

This paper presents the sterol composition of three species of pelagic to meso-

pelagic euphausiids, one species of a bathypelagic marine mysid and one species of an estuarine mysid, two species of pelagic marine copepods and one species of a bathypelagic marine copepod. The paper compares the sterol composition with those of other crustaceans in the marine food web and with phytoplankton and attempts to draw some conclusions as to whether the composition of the crustacean sterols is controlled metabolically by the animal as a result of its own specific physiological or biochemical requirements, or merely represents the type of dietary input, the animal having little control over its sterol make-up.

Material, Methods and Results

The animals were caught by net during biological cruises of *R.R.S. Discovery* to the northeastern Atlantic. They were sorted immediately by hand and where possible were kept alive for 3 h in clean, filtered sea water at 8 °C under low light conditions so that they might clear their guts. They were then frozen under nitrogen at −25 °C in specially cleaned glass jars. They were kept frozen at −25 °C until analysis.

Total lipids were extracted from the animals and the free sterol and sterol ester fractions crudely separated by methods previously described (Ballantine *et al.*, 1975, 1976). These fractions were then saponified with 10% potassium hydroxide in ethyl alcohol for 24 h under nitrogen in the dark and the non-saponified fraction purified by preparative TLC. The separated sterols which originated from the free and bound fractions were then determined using an SE30 0.3% textured glass bead column (Table I).

The sterols were identified and their relative amounts determined by the GLC of their trimethylsilyether (TMSE) derivatives on 1% Dexsil 300 GC and 1% Silar 5CP (2.5 m × 4 mm i.d. at 260 and 220 °C, respectively). These columns were coupled

Table I

Details of samples.

Species	Position caught	Depth of net (m)	Total lipid (% wet wt)	Sterol (% total lipid)	Sterol este (% total lipi
Meganyctiphanes norvegica M. Sars	25 °N; 17 °W	250–0	1.9	3.4	0.007
Thysanopoda subaequalis Boden	20 °N; 23 °W	1000–0	1.2	4.4	0.036
Stylocheiron abbreviatum G.O. Sars	25 °N; 17 °W	250–0	1.7	3.3	–
Eucopia sculpticauda Faxon	37 °N; 25 °W	1000–950	9.7	0.6	0.002
Neomysis integer (Leach)	Totton Marsh (Southampton)	surface	1.9	4.3	0.012
Labidocera acutifrons (Dana)	20 °N; 22 °W	surface	1.3	3.6	0.026
Calanus finmarchicus (Gunnerus)	60 °N; 20 °W	surface	2.7	0.7	–
Bathycalanus sp.	38 °N; 28 °W	1258–1264	7.7	0.2	–

through a silicone membrane separator to a fast scanning MS9 mass spectrometer, as previously described (Ballantine *et al.*, 1975). The results of the GLC-MS analysis upon the TMSE are tabulated in Tables II and III.

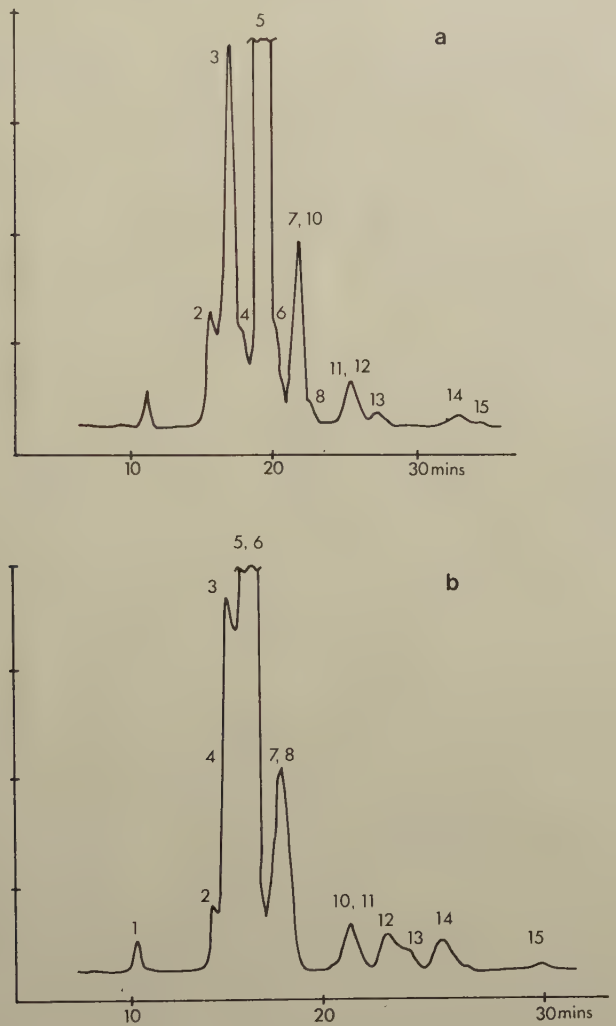

Fig. 1. Sterol profile of *Labidocera acutifrons* on Dexsil 300 GC (a) and Silar 5CP (b).

Fig. 1 represents the sterol profile of *Labidocera acutifrons*[1], which has been selected as an example because it contained almost all of the marine sterols found in the pelagic marine crustaceans. The TMSE derivatives of *L. acutifrons* were observed

[1] For taxonomic authorities see Table I.

TABLE II

Sterol composition of samples: tr, trace.

Species	Sterols[a]															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Meganyctiphanes norvegica	–	–	0.6	–	98.2	–	–	–	–	–	0.2	0.2	0.3	0.6	–	–
Thysanopoda subaequalis	–	–	1.1	–	96.6	–	–	–	tr	–	0.2	0.5	0.5	1.1	–	–
Stylocheiron abbreviatum	–	–	1.7	–	96.0	–	–	–	2.1	tr	tr	tr	tr	tr	–	–
Eucopia sculpticauda	–	–	0.5	–	96.1	–	–	–	–	–	0.5	0.8	1.1	1.1	–	–
Neomysis integer	–	–	3.2	–	88.0	–	0.8	–	–	1.0	1.0	2.9	0.6	2.4	tr	–
Labidocera acutifrons	0.7	1.4	14.3	tr	73.2	tr	4.2	0.6	–	1.2	0.3	1.6	0.9	1.6	tr	–
Calanus finmarchicus	2.7	1.5	14.1	tr	45.4	–	3.0	–	–	27.7	1.5	–	–	2.0	–	2.2
Bathycalanus sp.	–	–	16.9	–	80.5	–	–	–	–	–	0.2	0.5	0.3	1.5	–	–
Thalassionema nitzochioides (Ballantine *et al.*, 1979b)	1.5	tr	22.7	–	59.6	–	3.6	–	–	5.0	2.9	2.2	–	2.0	0.8	–

[a] *Key list for sterols*

Sterol	1	26C5,22E	22(E)-24-norcholesta-5,22-dien-3β-ol
	2	27C5, 22E*	occelasterol
	3	27C5,22E	22(E)-cholesta-5,22-dien-3β-ol
	4	27C,22E	22(E)-5α-cholest-22-en-3β-ol
	5	27C5	cholesterol
	6	27C	5α-cholestan-3β-ol
	7	28C5,22E	22(E),24(ε)-24-methylcholesta-5,22-dien-3β-ol
	8	28C,22E	22(E),24(ε)-24-methyl cholest-22-en-3β-ol
	9	27C5,7	cholesta-5,7-dien-3β-ol
	10	27C5,24(25)	desmosterol
	11	28C5	24(ε)-24-methylcholest-5-en-3β-ol
	12	28C5,24(28)	24-methylene cholesterol
	13	29C5,22E	22(E),24(ε)-24-ethylcholesta-5,22-dien-3β-ol
	14	29C5	24(ε)-24-ethylcholest-5-en-3β-ol
	15	29C5,24(28)Z	24(28)-24-ethylcholesta-5,24(28)-dien-3β-ol
	16	Unknown	

as eight partly resolved peaks in both the Dexsil and Silar chromatograms. By multiple mass spectrometric scanning of each of these peaks it was established that 14 different marine sterols were present in the extracts and their retention times were determined.

TABLE III

GL-MS characteristics of the sterol TMSE derivatives of *Labidocera acutifrons*.

Sterols	Identity[a]	Dexsil RRT	Silar RRT	M^+	Characteristic fragment ions[b]
1	26C5, 22E	0.63	0.65	442	427, 372, 352, 337, 313, 281, 255, *129*
2	27C5, 22E[c]	0.84	0.88	456	441, 366, 351, 327, 255, 215, 213, *129*
3	27C5, 22E	0.89	0.93	456	441, 366, 351, 327, 255, 215, 213, *129*
4	27C 22E	0.93	0.90	458	443, 374, 346, 345, *257*, 217, 215
5	27C5	1.00	1.00	458	443, 368, 353, 329, 275, 255, 247, *129*
6	27C	1.04	0.97	460	445, 370, 355, 306, 305, 217, 216, *215*
7	28C5, 22E	1.10	1.22	470	455, 380, 365, 341, 337, 255, 129, *125*
8	28C 22E	1.14	1.09	472	457, 374, 346, 345, *257*, 217, 215
10	27C5 24(25)	1.10	1.34	456	441, 366, 351, 343, 327, 255, 253, *129*
11	28C5	1.29	1.32	472	457, 382, 367, 343, 289, 261, 255, *129*
12	28C5, 24(28)	1.29	1.43	470	455, 386, 380, 365, 343, 341, 257, *129*
13	29C5, 22E	1.36	1.49	484	469, 394, 379, 355, 351, 255, 139, *129*
14	29C5	1.61	1.61	486	471, 396, 381, 357, 275, 255, 213, *129*
15	29C5, 24(28)Z	1.69	1.92	484	386, 371, 296, 281, 257, 255, 213, *129*

[a] For key see Table II.
[b] Only ions above m/z 125 are quoted in this table and the base peak is italicized.
[c] Occelasterol.

The sterols were identified (Table III) by a combination of their GLC retention times on the two different liquid phases and their characteristic mass spectrometric fragmentation patterns, which were compared with the GC-MS data for authentic sterol TMSE derivatives and for TMSE derivatives of known marine sterols obtained from other marine organisms as previously described (Ballantine *et al.*, 1975, 1976, 1977, 1978, 1979a,b). It must be noted that neither the GLC nor MS analysis is able to determine the stereochemistry at C24 in the sterol side chain and there was insufficient sample available to determine the stereochemistry by other means.

In this way sterols 1, 3, 5, 6, 7, 10, 11, 12, 13, 14 and 15 were identified (Tables II and III) as common sterols which were present in many marine organisms. Sterol 2 was identified as occelasterol obtained from an annelid by Kobayashi & Mitsuhaski (1974) and from the jellyfish *Pyrosoma* sp. by Ballantine *et al.* (1977). Sterol 4 was identified as 22-dehydrocholestanol and Sterol 8 was neospongesterol (or its C_{24} epimer) both of which were isolated by Erdman & Thomson (1972) from the sponge *Hymeniacidon perleve* and which were shown to be present in jellyfish (Ballantine *et al.*, 1975) and tunicates (Ballantine *et al.*, 1977). Sterol 9 was a minor sterol in the extracts of *Stylocheiron abbrevicatum* and was easily identified as cholesta-5,7-dien-3β-ol by comparison with authentic sterol.

The sterols were determined by GLC using the two liquid phases. The flame ionization detector response factors for a series of standard marine sterols on these phases were determined and found, within experimental error, to be identical. Each sterol in a mixture was located by mass spectrometry within the peaks of the chromatograms, and the peak areas of the various regions of the chromatograms were determined by cutting out and weighing on an analytical balance. By comparing the areas containing each individual sterol and by using subtractive techniques from the results of the two different columns, the relative amounts of each sterol could be determined. Cross-checks using different column systems were employed and the results averaged. In general, there was good agreement in the cross-correlations and the results have an estimated error range of the order of $\pm 2\%$ for abundant sterols and $\pm 0.3\%$ for minor sterols.

Discussion and Conclusions

Before any discussion of the results it should be pointed out that although a period was allowed for the animals used to clear their guts prior to sacrifice, "trace" sterols detected in the animals could be coming from residual, non-absorbed gut contents.

The predominance of cholesterol in the euphausiids is in agreement with the findings of Idler & Wiseman (1971) for *Nyctiphanes norvegica*. The minor sterols they found in the mixture were, however, 22(E)-cholesta-5,22-dien-3β-ol, 22(E),24(ε)-24-methylcholesta-5,22-dien-3β-ol (0.1%), desmosterol (0.4%), and three unknown sterols. This result is at variance with the present work. It is possible, however, that since Idler and Wiseman used a single column to determine the sterol composition of the animal, the desmosterol they found was in fact cholesta-5,7-dien-3β-ol as the column (3% XE. 60-3% NGS) does not resolve these two sterols (desmosterol RRT = 1.29 and 7-dehydrocholesterol RRT = 1.30).

The predominance of cholesterol in the mysids is in agreement with the findings of Teshima & Kanazawa (1971) but the large quantities of 22(E),24(ε)-methylcholesta-5,22-dien-3β-ol and methylenecholesterol reported by these authors to be present in *Neomysis intermedia* were not found in the species examined in the present work. The bathypelagic species *Eucopia sculpticauda* was found to have a relatively low percentage of sterol in its lipids but this was due to the large amount of wax esters in its total lipid (e.g. Morris, 1972).

In agreement with the results of Lee *et al.* (1974) with the species *Euchaeta japonica*, the copepod *Calanus finmarchicus* analysed in the present work also contained a large proportion of desmosterol (27.7%). This sterol was, however, either absent or present as a minor component in the other two copepod species analysed.

The majority of the other copepod sterols, 1, 2, 3, 4, 6, 7, (8,9), 11, 12, 13(14), and 15 reported in this work are new to the copepods and the stanols, 4, 8, and sterol 2 are new to the crustaceans as a group.

The three euphausiid species examined in this work are generally found in different geographical areas and at different environmental depths and hence are thought to occupy rather different trophic levels. *Meganyctiphanes norvegica* and *Thysanopoda subaequalis* almost certainly feed for part of their time on mixed phytoplankton and smaller zooplankton in the euphotic zone whilst *Stylocheiron abbreviatum* does not normally enter the upper euphotic zone. From our results it would appear that irrespective of trophic level these euphausiids have developed an almost uni-specific requirement for cholesterol as their body sterol. Only the macrurid decapods (unpubl. data), to which euphausiids are fairly closely related, and possibly the bathypelagic mysids (this work, see later) amongst the crustaceans have a similar sterol composition, completely dominated by cholesterol.

In contrast, the results for the mysids together with the data of Teshima & Kanazawa (1971) show a considerable variation in sterol composition. The carnivorous bathypelagic *Eucopia* species (see Table II) has the same cholesterol-dominated composition as the euphausiids but the two surface omnivorous *Neomysis* species (see Table II and Teshima & Kanazawa, 1971) have a far more diverse composition with 22-dehydrocholesterol, brassicasterol (or crinosterol), methylene cholesterol, and β-sitosterol being important sterol components.

The results of the copepod analyses indicate that the two near-surface species contain a significantly greater variety of sterols than their bathypelagic counterpart *Bathycalanus* sp. For example sterols 1, 2, 4, 6, 7, 8, and 10 are present in the omnivorous surface species *Labidocera acutifrons* but not in the carnivorous bathypelagic species. It is now accepted that members of the Arthropoda cannot biosynthesize sterols in vivo but can convert ingested phytosterols to cholesterol via a desmosterol intermediate (e.g. Thompson *et al.*, 1972). Thus, the sterols observed in the surface species are probably of either exogenous origin or are modifications of sterols of exogenous origin. Unfortunately little is known of the inter-relationships between sterols of natural field populations of phytoplankton and zooplankton due to the difficulties involved in obtaining field samples containing only one species (see discussion in Ballantine *et al.*, 1979b). It seems probable however, that the large quantity of "phytosterol" produced by the phytoplankton in the upper reaches of the sea is converted by the crustacean members of the plankton into cholesterol. Because the phytoplankton are found in the upper 50 m or so of the oceans then one would expect the surface crustacean species to contain the greatest number of sterols. Certainly most pelagic copepods will be omnivorous (Marshall, 1973), and *Calanus finmarchicus* and *Labidocera acutifrons* are thought to be predominantly herbivorous.

In comparing the published sterol analyses of phytoplankton cultures with the present data, it is essential to consider the possible effects of culture condition and stage of growth in the composition of the phytoplankton (see Ballantine *et al.*, 1979b). Therefore, we have mainly compared the copepod data with the published sterol composition of a single species of marine diatom taken from a single natural

field population in the northwestern Atlantic, i.e. the same general area from which the copepods were taken (Ballantine *et al.*, 1979b). In detail, the sterol composition of *Labidocera acutifrons* is very similar to that found in the free-living diatom *Thalassionema nitzschioides* (see Table II) although cholesterol is a more major component of the copepod sterols and stanols were not found in the diatom. The surface calanoid species (*Calanus finmarchicus*) contains many of the sterols found in phytoplankton and its major sterols, 22-dehydrocholesterol, cholesterol, and desmosterol are also the major sterols of the free-living diatom, although they are present in different relative amounts – desmosterol in particular appearing to be of importance to this copepod. The deep-water calanoid species (*Bathycalanus* sp.) has a much simpler sterol composition than is normally found in phytoplankton with 22-dehydrocholesterol and cholesterol comprising over 97% of the component sterols. The complete absence of desmosterol in this species compared with its presence as a major sterol in the surface calanoid species may indicate a particular requirement for desmosterol in *Calanus finmarchicus*.

The results suggest that many of the sterols found in the surface copepods may be of a dietary origin although the animals have clearly modified them to a varying degree, presumably to suit their particular needs. The presence of stanols in these species but not in marine diatoms (Ballantine *et al.*, 1979b) which are likely to form part of their diet, may indicate that either the stanols are intermediates involved in the conversion of phytosterols to sterols required by the copepods in vivo or that they are being synthesized by the copepods for specific physiological-biochemical purposes.

Acknowledgements

The authors gratefully acknowledge support from the Institute of Oceanographic Sciences in the form of a research contract (NERC F60/B1/6), the Science Research Council for a studentship to one of us (J. C. R.), and the help of colleagues in the Biology Department of the I.O.S. in the collection and identification of the animals used in this study.

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THE RÔLE OF TEMPERATURE AND DAYLENGTH IN THE CONTROL OF THE REPRODUCTIVE CYCLE OF *HARMOTHOE IMBRICATA* (L.) (POLYCHAETA: POLYNOIDAE)

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Abstract: Both temperature and daylength affect the rates of various processes in the gametogenic cycles of the two sexes of *Harmothoe imbricata* (L.), but there is no evidence for any environmental trigger to spawning. Control of the spawning season is, therefore, dependent on the control of the timing of events leading to the production of mature gametes. An internal clock mechanism, rather than environmental conditions, provides this control, and it also serves to synchronize events between individuals in the population. It is likely that daylength provides the necessary exogenous input (Zeitgeber) to the internal rhythms of individual animals. In view of the strong correlation between the direction of change in temperature and daylength in the field, and the direction in which these factors tend to favour the processes of gametogenesis, it is proposed that natural changes in these two variables will tend to act synergistically with the internal mechanism to synchronize individuals throughout gametogenesis.

INTRODUCTION

Harmothoe imbricata (L.) is the commonest polynoid at Cullercoats, Tyne and Wear, U.K., and its reproductive cycle at this locality is known in some detail (Daly, 1972, 1974; Garwood, in prep.). It is a spring breeder, with a spawning season extending from late February until the beginning of May, during which each female releases two batches of oocytes. The duration of the spawning season, and the timing of the two peaks of activity within it, are remarkably constant from year to year, and gametogenesis proceeds in a relatively well synchronized manner within the population. This implies a strict temporal control of the events leading up to the attainment of sexual maturity, and this paper outlines an investigation into the factors responsible for controlling this annual reproductive cycle.

MATERIALS AND METHODS

Individual animals, collected from the intertidal zone to the north of Cullercoats Bay, Tyne and Wear, were maintained in the laboratory in plastic beakers containing ≈ 100 ml of sea water. Each was provided with an empty *Patella* shell,

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on or underneath which the animal could normally be found. Pieces of the spionid *Malacoceros fuliginosus* (Claparède) were provided once or twice a week as food, and the water in the beakers was changed the day after each feeding. The beakers were kept in tanks of tap water, circulated by Churchill Thermochiller units, which maintained them at the appropriate temperature. Light was supplied by 15-W tungsten filament light bulbs, operated by Sangamo time switches.

The operative technique consisted of the removal of a length of segmental blood vessel, with germ cells attached, from a fertile segment, through a slit made at the base of a parapodium, after narcotizing the animal in 0.07% MS222 (Sandoz) in sea water. The early stages of gametogenesis were studied by means of acetic-orcein squashes of the cells on the blood vessel, made permanent by the dry ice method of Conger & Fairchild (1953). Growth of the first cohort of oocytes was followed in terms of the increase in modal oocyte diameter. Blood vessel, with oocytes attached, was left overnight in a dissociating fluid (a mixture of 1% Lugol's Iodine and saturated boric acid solution, in the ratio 1 : 4), and the sample was then teased apart on a microscope slide. The larger diameter of each oocyte was measured and assigned to one of a series of 7.5-μm size classes, until one such class contained 15 oocytes. The mid-point of that class was taken as the modal diameter for that animal, and used in the subsequent calculation of modal volume.[1] Oocyte growth rates were calculated according to the formula:

$$K = \frac{\ln V_2 - \ln V_1}{t_2 - t_1},$$

where K is the instantaneous relative growth rate, V_1 is the modal oocyte volume at time t_1, and V_2 is the modal oocyte volume at time t_2 (Brody, 1945).

To follow the growth of the second cohort of oocytes, a different approach was adopted, involving the measurement of the time interval between the two spawnings of an individual female. It is assumed that this inter-spawning interval is inversely proportional to the rate of oocyte growth.

For the study of the timing of spawning, heterosexual pairs were set up as the females approached maturity. These were maintained in larger plastic containers, in the same way as individual animals. Samples of coelomic fluid were removed from males using a micro-capillary, and by examining the stages of maturation present, the date of release of spermatocytes into the coelom was estimated, using the time-table of gamete maturation in males set out by Daly (1972).

[1] This is not strictly the modal volume as it is based on equal diameter rather than equal volume classes.

Results

It is possible to recognize a well-defined series of stages in the reproductive cycles of the two sexes of *Harmothoe imbricata* (Daly, 1972, 1974; Garwood in prep.). These are as follows: females, (1) the onset and early processes of oogenesis, (2) vitellogenesis in the first cohort of oocytes, (3) the first spawning, (4) vitellogenesis in the second cohort, (5) the second spawning, and (6) the resting phase; males, (1) the onset and early processes of spermatogenesis, (2) the release of spermatocytes into the coelom, followed by the meiotic divisions and maturation to spermatozoa, (3) spawning, and (4) the resting phase.

The annual cycles of temperature and daylength upon which the experiments reported here were based are illustrated in Fig. 1, which also shows their relationship to the stages in the reproductive cycles of the two sexes. The temperature curve is the average, over a number of years, of the mean monthly sea temperature measured at the inlet to the circulating sea water system at the Dove Marine Laboratory, Cullercoats, and the hours of light per day were based on the times of sunrise and sunset at Liverpool.

THE EARLY STAGES OF GAMETOGENESIS

In the shore population, germ cell proliferation begins in August (Garwood, in prep.), when temperature is near its maximum and as the days are getting shorter (Fig. 1). By examining the types of cells attached to the blood vessels of fertile segments, it was possible to decide whether or not gametogenesis had begun in an individual, and on 25 June 1977 an experiment was started to investigate the possibility

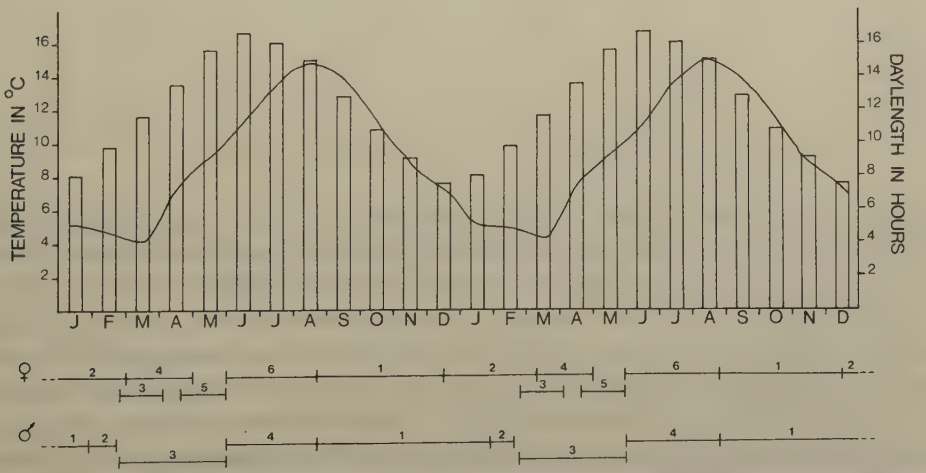

Fig. 1. The relationship between the stages of the reproductive cycles of the two sexes (as described above) and the annual cycles of temperature (solid line) and daylength (vertical columns).

of environmental influence on this phase. The daylength and temperature regimes used are given in Table I. Regime 1, which effectively brings animals into winter temperature conditions in the middle of summer, was designed to test whether high temperature was a prerequisite for the onset of proliferative activity, and the other two regimes attempted to separate any possible influences of temperature and daylength.

TABLE I

Environmental conditions used for the investigation of the control of the onset of gametogenesis.

Group	Temperature	Daylength
1	Constant, 4 °C	Following a simulated ambient cycle
2	Following a simulated cycle 2 months behind the ambient cycle	Following a simulated cycle 2 months ahead of the ambient cycle
3	Following a simulated cycle 2 months behind the ambient cycle	Following a simulated cycle 2 months behind the ambient cycle

TABLE II

The numbers of animals in samples taken from the shore population and from each of the experimental groups, in which gametogenesis had begun.

	Shore			Group 1			Group 2			Group 3		
Date	Yes	?	No	Yes	?	No	Yes	?	No	Yes	?	No
1 June	0	0	8	–	–	–	–	–	–	–	–	–
16 June	–	–	–	0	0	3	0	0	3	0	0	3
30 June	0	0	4	–	–	–	–	–	–	–	–	–
5 Aug.	–	–	–	0	0	3	0	1	2	0	1	2
26 Aug.	2	0	3	1	1	1	3	0	0	3	0	0
9 Sept.	3	2	0	3	0	0	3	0	0	3	0	0

The results of this experiment are summarized in Table II, together with some parallel observations on the shore population. Despite the small numbers of animals involved, it is clear that there was no sign of proliferation of germ cells in any of the groups before the beginning of August, and by September gametogenesis was under way in all the animals in each sample. This suggests that environmental conditions, at least in the 2 months prior to the onset of gametogenic activity, do not influence the timing of this phase. More specifically, the experiment shows that gametogenesis will begin under a wide range of temperature (4–14 °C) and daylength (10L : 14D to 16L : 8D) conditions. These two ranges represent almost the entire annual ranges of these two variables.

The early processes of gametogenesis in females consist of protogonial and oogonial divisions, followed by maturation of oogonia through the various pre-meiotic stages and their accumulation as previtellogenic oocytes. It occupies the period from the onset of oogenesis in August until the beginning of the vitellogenic growth phase in the first cohort of oocytes in December. Its starting date was arbitrarily set at 20 August, and its end was taken as the time when the combined oocyte diameter frequency shows a peak at 50 μm. From Fig. 1 it can be seen that this stage begins as daylength is decreasing and when sea temperature is around its maximum, and continues as both these factors decrease.

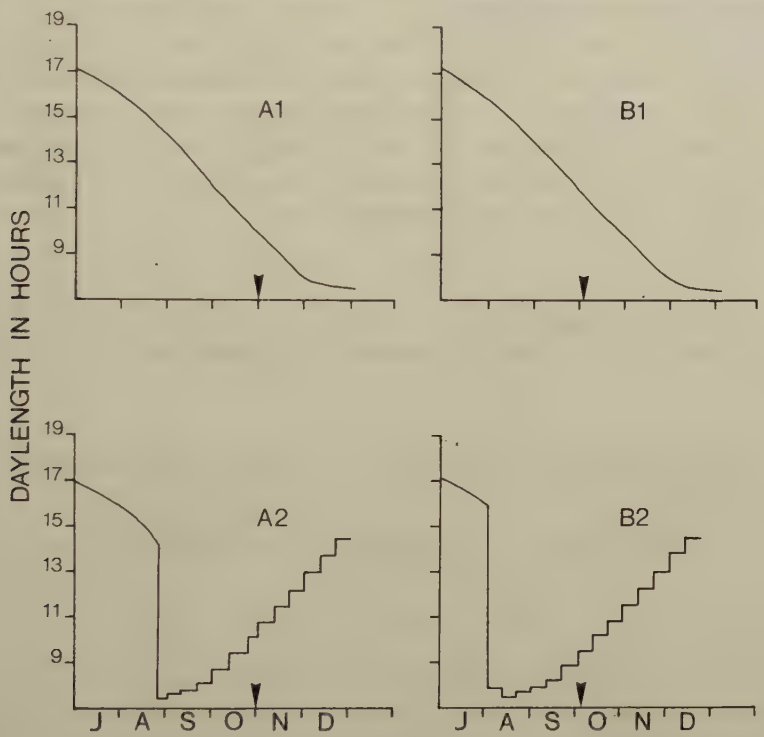

Fig. 2. The influence of daylength on the end-point of the early processes of oogenesis (arrowed): Groups A1 and B1 were kept under ambient daylength conditions, while Groups A2 and B2 were subjected to an artificial increasing daylength regime.

A preliminary experiment, the results of which are shown in Fig. 2, indicates that daylength has no effect on the duration of the early processes of oogenesis. Two pairs of groups of animals were kept in the laboratory from the time of the onset of gametogenesis, the members of each pair (A1 and A2, B1 and B2) being maintained under identical temperature conditions. One group of each pair (A1 and B1) were kept under ambient daylength conditions, whilst the other (A2 and B2) was subjected to

an artificial increasing daylength regime, as indicated in Fig. 2. The latter two groups were, therefore, given daylength conditions inappropriate for the time of year, when daylength is normally decreasing. This resulted in them being given a considerably smaller mean daily daylight-hour dose (total daylight-hours divided by the duration of the phase in question – a measure of the average daylength to which they were exposed) of ≈ 9 h per day compared with 12.5 h for the two groups under ambient daylengths. Despite these differences in daylength conditions, there were no differences in the duration of the early processes of oogenesis between the groups making up a pair (i.e. between A1 and A2, B1 and B2), as shown by the date when the combined oocyte diameter frequencies reached a peak at 50 μm.

The differences between the pairs (Fig. 2) are due to the differences in the temperature conditions they experienced, and this factor was found to be very important in determining the duration of the early stages of oogenesis. By keeping animals at a constant low temperature, it was possible to advance the date of entry of oocytes into the first wave of vitellogenesis by some $2\frac{1}{2}$ months (Table III). The relationship between the duration of the early processes of oogenesis (y) and the mean daily temperature dose (x) is governed by the expression:

$$y = 16.2x - 30.7.$$

The higher the temperature dose, the longer the stage lasts, while at a constant temperature of 4 °C, it is reduced to one-third of its normal length.

TABLE III

The duration of the early processes of oogenesis under different temperature conditions.

Mean duration (days)	Number of animals	Mean daily temperature dose (°C)
130	shore	10.4
120	13	8.9
97	7	7.3
72	18	6.4
40	17	4.0
35	12	4.9

In males, the early processes of spermatogenesis continue from August until late January or early February, when the germ cells are released into the coelom, and includes both the divisions of protogonia and spermatogonia, and the maturation of spermatogonia to spermatocytes. This stage continues as temperature drops from its maximum to its minimum value, and as daylength decreases to its minimum and begins to increase again (Fig. 1). The duration of the early processes of spermatogenesis is measured from the onset of gametogenesis (20 August) until the date of release of spermatocytes into the coelom.

As was found in females, the early stages of gametogenesis in males are profoundly affected by temperature conditions, being shorter under low temperature conditions. In contrast to the situation in females, however, this stage in males is also influenced by daylength conditions. The results of experiments, summarized in Table IV, were used to generate the following equation governing the relationship between

TABLE IV

The relationship between the duration of the early stages of spermatogenesis and the temperature and daylength conditions under which the animals were maintained.

Mean duration (days)	Number of animals	Mean daily temperature dose (°C)	Mean daily daylight-hour dose (h)
167	14	9.13	10.45
164	16	7.5	10.1
160	13	9.5	10.2
160	10	9.5	10.2
160	11	9.5	10.7
156	9	9.4	12.1
155	8	9.5	11.4
154	8	10.4	10.7
149	11	5.0	12.0
133	9	4.0	11.0
128	5	5.6	11.2
114	5	5.2	11.2
112	5	5.6	10.1
100	5	5.5	9.8

the duration of the stage in days (y) and the mean daily temperature (x_1) and daylight-hour (x_2) doses:

$$y = 7.17x_1 + 6.54x_2 + 18.29.$$

Both partial regression coefficients are significantly different from zero (SE 1.83 and 4.42, respectively), indicating that the variation in both factors is contributing to the variation in the duration of the stage. As both coefficients are positive, the early processes of spermatogenesis proceed most rapidly under low temperature and short daylength conditions.

MATURATION

Vitellogenesis in *H. imbricata* occurs in two successive waves, the first cohort of oocytes beginning to grow in mid-December, to be spawned in late February or March. A second cohort then starts to develop, with spawning following about 30 days later (see p. 37, Fig. 1). These two waves are quite distinct and will be considered separately.

The first wave of vitellogenesis begins at around the minimum daylength and proceeds as it increases, and at the same time, temperature shows a steady decline (Fig. 1). A preliminary experiment was designed to test the influence of daylength on the rate of growth of the first cohort of oocytes, under constant temperature conditions. At the end of December 1975, two groups of eight females were brought into the laboratory, and maintained at 4 °C. One group followed the ambient daylength curve (see Fig. 1), while the other was subjected to a constant 13L : 11D regime. This represents a slightly longer day than the maximum normally experienced during this stage, and results in a considerably higher mean daily daylight-hour dose of 13 h per day, compared with 9.3 h for those under ambient daylengths.

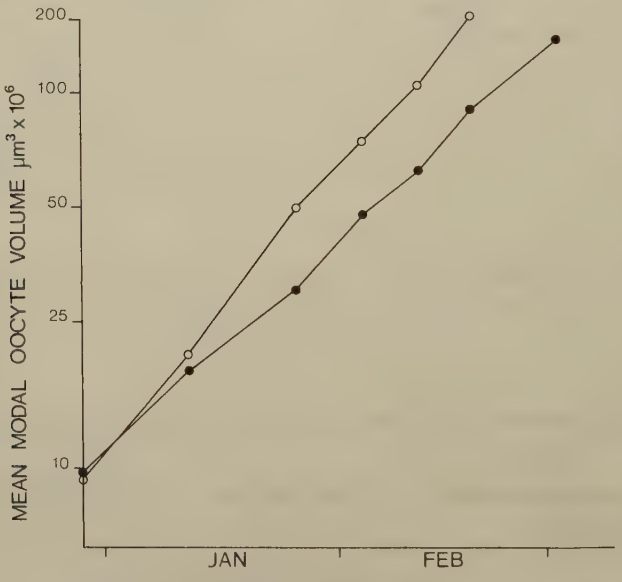

Fig. 3. Growth of the first cohort of oocytes at 5 °C, under ambient daylength conditions (●) or under a constant 13L : 11D regime (○).

The increase in mean modal oocyte volume for the two groups is shown in Fig. 3, and the two curves are seen to diverge. The mean overall instantaneous relative growth rate (calculated from initial and final modal oocyte volumes) is significantly larger for animals under the 13L : 11D regime than for those under ambient daylength conditions (0.056 ± 0.007 compared with 0.044 ± 0.010, $t = 7.98$, $P = 0.01$, t-test for small sample size, Bailey, 1973). Growth of the first cohort of oocytes is, therefore, accelerated by moderately long daylengths.

In a more extensive experiment, involving the manipulation of both temperature and daylength conditions, a series of seven regimes was used. At each of three constant temperatures (5, 10, and 15 °C), one constant daylength regime (12L : 12D),

and one regime following the ambient daylength curve (see Fig. 1) were used. An additional constant 16L : 8D regime was used at 5 °C only. Fig. 4 shows the change in mean modal oocyte volume for each group, plotted on a semi-logarithmic scale and considered under the headings 5, 10 and 15 °C, ambient light, and 12L : 12D for convenience. Mean overall rates of oocyte growth for the seven groups were compared using an SNK ranked analysis of variance (Sokal & Rolf, 1969), which showed that growth of the first cohort of oocytes was fastest at 5 °C, slower at 10 °C, and at 15 °C growth was negative, due to the degeneration of large oocytes. At both 5 and 10 °C, growth was faster under the 12L : 12D regime than under ambient daylengths, although daylength had no effect at 15 °C. Under the long daylength (16L : 8D) conditions at 5 °C, growth was faster than under ambient conditions, but not significantly different from that under the 12L : 12D regime.

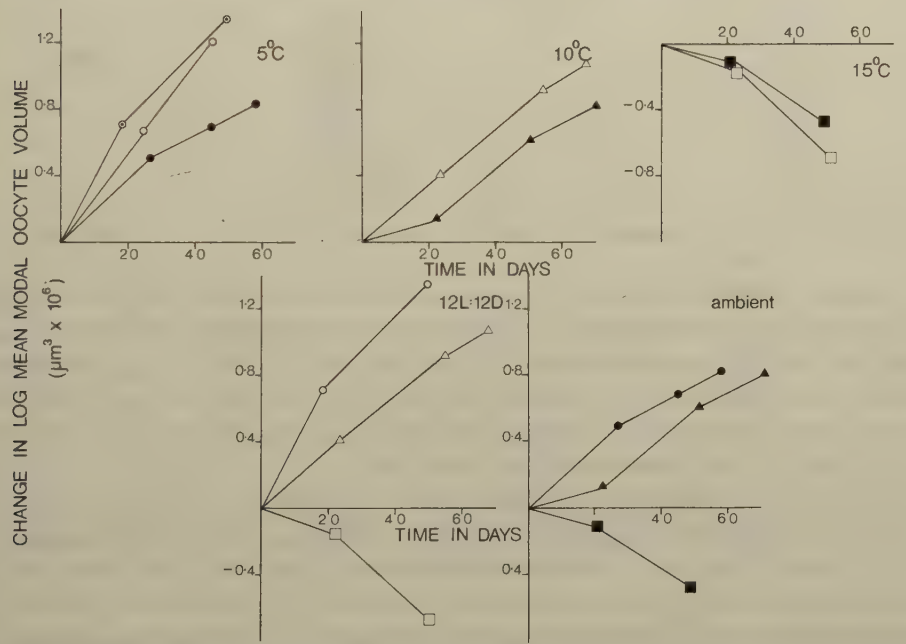

Fig. 4. Growth of the first cohort of oocytes under various combinations of temperature and daylength conditions:

○ ● ⊙ 5 °C
△ ▲ 10 °C
□ ■ 15 °C
● ▲ ■ ambient daylength
○ △ □ 12L : 12D
⊙ 16L : 8D

The results of both experiments on the influences of temperature and daylength on the rate of growth of the first cohort of oocytes are summarized in Table V, and the relationship between these three variables is governed by the following equation:

$$y = 44.3 - 6.3x_1 + 3.48x_2,$$

where y is the mean overall rate of growth of oocytes, x_1 is the mean daily temperature dose, and x_2 is the mean daily daylight-hour dose. Both partial regression coefficients are significantly different from zero (SE 0.91 and 1.74, respectively) and their signs indicate that growth of the first cohort of oocytes proceeds most quickly under low temperature and long daylength conditions.

TABLE V

The rate of growth of the first cohort of oocytes under various temperature and daylength conditions.

Mean overall growth rate ($\ln \mu m^3 \cdot day^{-1}$)	Number of animals	Mean daily temperature dose (°C)	Mean daily daylight-hour dose (h)
0.069	9	5	16
0.061	11	5	12
0.056	8	4	13
0.044	9	4	9.3
0.043	8	6.6	9.3
0.039	10	10	12
0.036	11	5	9.5
0.027	14	10	9.8
−0.022	7	15	12
−0.022	9	15	9

It is clear, however, that such an interpretation of the control of growth in the first cohort is an over-simplification. The influence of the two environmental factors is not linear over the range investigated. The accelerating influence of longer daylength begins to tail off somewhere between 13 and 16 h per day, and at a temperature between 10 and 15 °C growth is no longer possible. The regression equation presented above does not take into account such limitations.

In addition to temperature and daylength, a third factor, the time of entry of oocytes into vitellogenesis, was found to play an important rôle in determining the rate of oocyte growth. It has been shown (see p. 40) that this point can be brought forward by up to $2\frac{1}{2}$ months in the laboratory, by maintaining animals at low temperature during the early processes of oogenesis. Under these circumstances, the subsequent rate of growth of the oocytes cannot be explained simply in terms of the temperature and daylength conditions. Under constant or decreasing daylength conditions (the latter representing ambient conditions for the time of year, but not the conditions under which this stage normally occurs; see Fig. 1), at a temperature of 4–5 °C, growth of oocytes is considerably slower in such animals, compared with those developing oocytes at the normal time of year (Fig. 5). The accelerating influence of longer daylengths, described above, is found to be reversed in animals induced to grow their oocytes abnormally early, so that it is significantly slower at 16L : 8D than at 12L : 12D.

This slower rate of growth of oocytes associated with early entry into vitellogenesis

can, however, be counteracted by subjecting the animals to a daylength regime simulating that when this stage is normally occurring, i.e. an increasing daylength regime (Fig. 6). The rate of oocyte growth under these conditions is as one would predict from the equation given on p. 43 knowing the temperature and daylength conditions to which the animals were exposed.

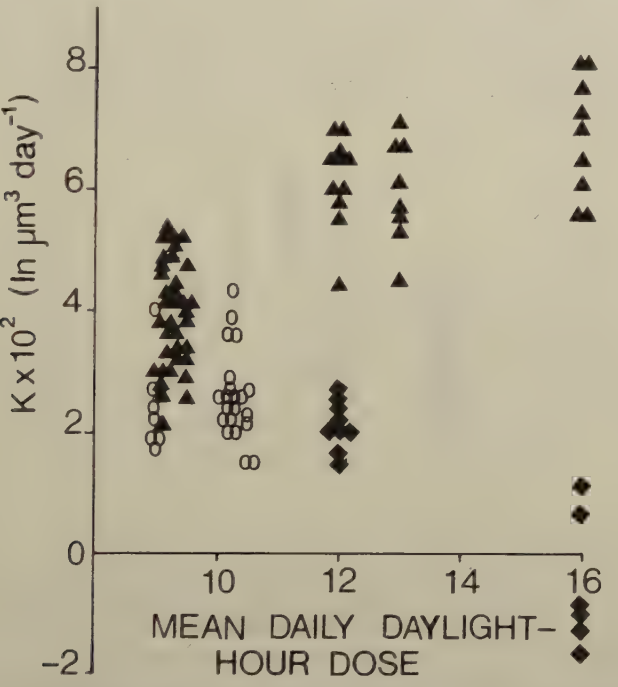

Fig. 5. The overall instantaneous growth rate of the first cohort of oocytes (K) in animals maintained at 5 °C under different daylength regimes: ▲, animals developing oocytes at the normal time of year; ○, animals developing oocytes early under decreasing daylengths; ◆, animals developing oocytes early under constant daylength conditions.

The growth of the second cohort of oocytes occurs in March and April, under increasing daylength and temperature conditions (Fig. 1). By transferring animals to the appropriate conditions as soon as they had completed their first spawning, the influences of temperature and daylength on the inter-spawning interval were investigated. As with growth of the first cohort of oocytes, both of these factors were found to affect the rate of development of the second cohort (Table VI). In marked contrast to the first cohort, however, growth of the second can occur at 15 °C, and indeed is faster at this temperature than at any of the others used (SNK ranked analysis of variance). There is also a slight tendency for short daylengths to slow down the rate of oocyte development, although it is only under very short daylength conditions (8L : 16D) that a significant increase in the inter-spawning interval is ap-

parent. In general, the rates of growth of the second cohort of oocytes are less sensitive to environmental conditions than are those of the first.

In males, the maturative stage of gametogenesis is short, lasting only 2–3 wk (Daly, 1972), from the time of release of spermatocytes into the coelom until the

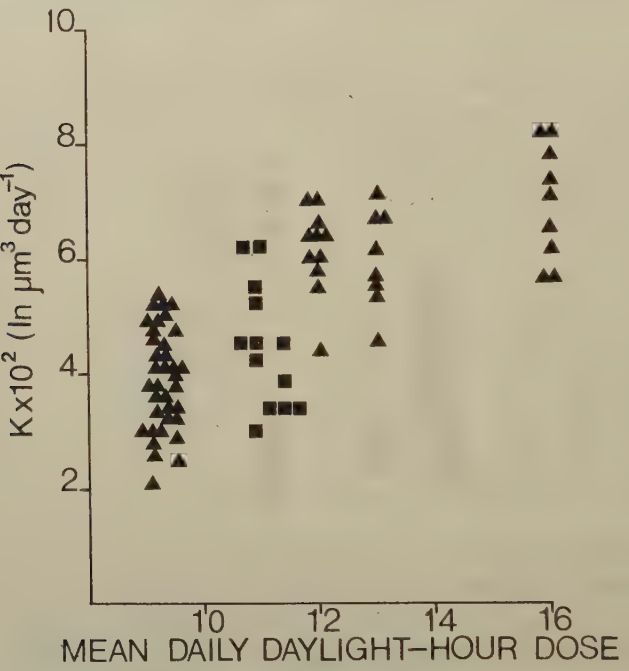

Fig. 6. The overall instantaneous growth rate of the first cohort of oocytes (K) in animals maintained at 5 °C under different daylength regimes: ▲, animals developing oocytes at the normal time of year; ■, animals developing oocytes early under an artificial increasing daylength regime.

TABLE VI

The mean duration of the inter-spawning interval in groups of animals maintained under various temperature and daylength conditions.

Mean inter-spawning interval (days)	Number of animals	Temperature (°C)	Daylength (h)
24.4	11	15	ambient
28.2	5	10	ambient
31.6	9	ambient	ambient
32.5	24	5	ambient
32.9	16	5	16L : 8D
33.3	7	5	13L : 11D
33.4	11	5	12L : 12D
35.9	8	5	ambient
37.9	17	5	8L : 16D

collection of mature spermatozoa into the nephridia. On the shore, this is in late January or February, when sea temperature is low (5–6 °C) and daylength is increasing from ≈9 to 10 h day (Fig. 1). As a consequence of the short duration of this stage, the rôles of environmental factors in the control of its rate could not be studied. The only relevant observation was that at a temperature of 10 °C and above, some abnormalities in the meiotic divisions were apparent. The elongation of the germ cells which normally takes place as the spermatid tetrads mature (Daly, 1974) occurs prematurely in a proportion of the cells in the coelom, so that those in the one- and two-cell stage were often mis-shapen. Nevertheless, animals kept at these temperatures developed enough normal spermatozoa to be able to fertilize a full batch of oocytes.

SPAWNING

At Cullercoats, *H. imbricata* spawns from the end of February until the beginning of May, each female releasing its two successive batches of oocytes ≈30 days apart. During the breeding season, temperature is increasing from its minimum of 4–5 °C to ≈8 °C, and daylength is steadily increasing from 10–14 h per day (Fig. 1).

In the laboratory, the first spawning has occurred at temperatures of 4–10 °C, and at daylengths of 8–16 h per day. The second spawning has been successful under a similar range of daylengths and a wider range of temperatures, up to 15 °C. These observations strongly suggest that there is no environmental trigger to spawning, and that it simply occurs as soon as oocytes have reached full size, been released into the coelom, and then collected into the nephridia. Any limits imposed by environmental conditions (such as the failure of spawning of the first batch of oocytes at 15 °C) can be explained in terms of an inhibition of the later stages of oocyte growth, rather than a direct effect on spawning itself.

The rôle of males in spawning is largely passive and Daly (1972) has shown that they release sperm in the presence of unfertilized eggs, fertilized ones producing no response. In the absence of a male, however, spawning in the female may be delayed by up to 10 days, in comparison with a paired female (Daly, 1972). The stage at which this delay is affected is not clear, and the validity of this observation when applied to the shore population is dubious, as unpaired females are rarely, if ever, found when they have oocytes approaching full size.

Although the spawning date is clearly not determined by an environmental trigger, there is evidence to suggest that spawning only ocurs during a restricted part of the day. During the 1975 spawning season, a large number of pairs of animals were maintained in the laboratory, and examined twice each day at ≈0900 and 1700 h for the presence of embryos under the elytra of the female. In not a single case were embryos first seen at the afternoon inspection, but always in the morning, indicating that spawning took place sometime between 1700 and 0900 h. Under constant light conditions, spawning did occur during the period of natural daylight,

after as little as three days. This suggests that the normal restriction of spawning to the hours of darkness is more likely to be the result of direct environmental input, rather than the manifestation of an endogenous circadian rhythm.

On the shore, the first spawning is normally from ≈26 February to 6 April. Fig. 7 summarizes the ranges of spawning dates, in the laboratory, of a number of

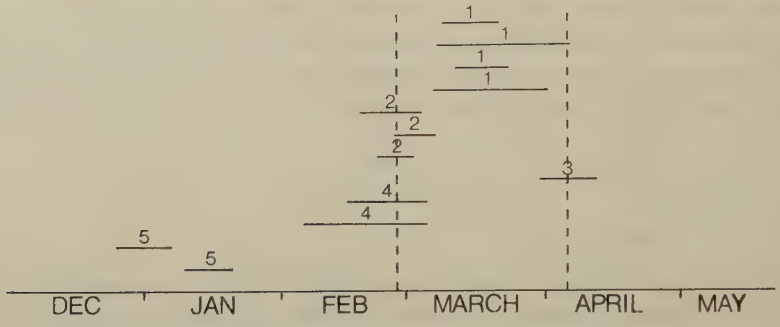

Fig. 7. The ranges of dates of first spawning in groups of animals maintained under a variety of conditions during gametogenesis: 1, conditions approximating those on the shore; 2, low temperature, long daylength conditions during vitellogenesis; 3, moderately high temperature (10 °C) during vitellogenesis; ambient or constant daylength conditions after early entry into vitellogenesis; 5, increasing daylength conditions after early entry into vitellogenesis.

groups of animals which had been subjected to a variety of conditions during gametogenesis. In animals kept under conditions approximating those on the shore during the vitellogenic period (Fig. 7, 1) spawning was well within the normal range, indicating that the artificial laboratory conditions adequately represented those on the shore. By subjecting animals to conditions more favourable for vitellogenic growth (long daylength and low temperature), spawning was advanced by ≈2 wk (Fig. 7, 2). Spawning was delayed by a comparable amount when animals were kept in conditions less suitable for vitellogenesis (at 10 °C) (Fig. 7, 3). Even in animals induced to enter vitellogenesis some $2\frac{1}{2}$ months in advance of normal, kept under ambient or constant daylength conditions during vitellogenesis (Fig. 7, 4), spawning is only brought forward by about 1 month. It is only if such animals are kept in an artificial increasing daylength regime during vitellogenesis (Fig. 7, 5) that the advancement in the date of entry into vitellogenesis is reflected in a pronounced advancement in the spawning date.

SYNCHRONIZATION WITHIN THE POPULATION

Olive (in press) has suggested that it is theoretically possible for environmental factors to synchronize gametogenic events between members of a population, if one important criterion is met. The direction of change of the environmental factor(s) concerned during a particular stage in gametogenesis must be the same as

that which will favour or accelerate that stage. In the particular case of the influence of temperature and daylength on the rate of development of the first cohort of oocytes in *H. imbricata*, this condition is met, and it is possible to test the hypothesis, as the degree of synchrony within a group of animals can be estimated at the beginning and end of the phase.

Under simulated ambient conditions, there is strong evidence of synchronization during this stage. When the log initial modal oocyte volume (a measure of the date of entry into vitellogenesis) is plotted against the subsequent rate of growth of oocytes (Fig. 8), there is a clear negative correlation. The larger the initial volume

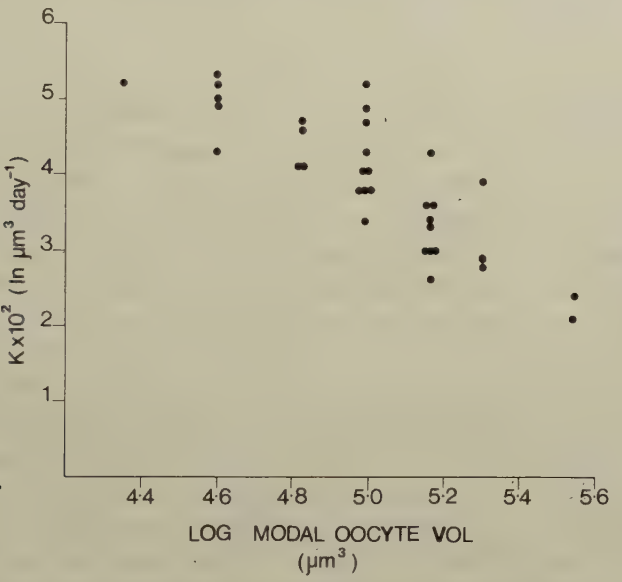

Fig. 8. Synchronization between individuals during the first wave of vitellogenic growth, under ambient conditions.

(i.e. the earlier the date of entry into vitellogenesis), the slower the rate of growth of oocytes in comparison with animals with small initial oocyte volumes (i.e. those entering this phase later). As the increase in log modal volume is ≈ linear (see Fig. 3), the correlation seen in Fig. 8 cannot be explained simply in terms of slower growth in the larger oocytes, and must represent real synchronization.

If this synchronization were a consequence of the effects of environmental factors on the rate of oocyte growth, then the correlation seen in Fig. 8 should not be seen in animals maintained under constant conditions. Fig. 9, however, shows that this is not the case, and that synchronization persists under a variety of constant conditions. This suggests that an endogenous mechanism is controlling the rate of oocyte growth, according to when the first wave of vitellogenesis was initiated. This effect is directly comparable to the generally slower rate of oocyte growth in animals

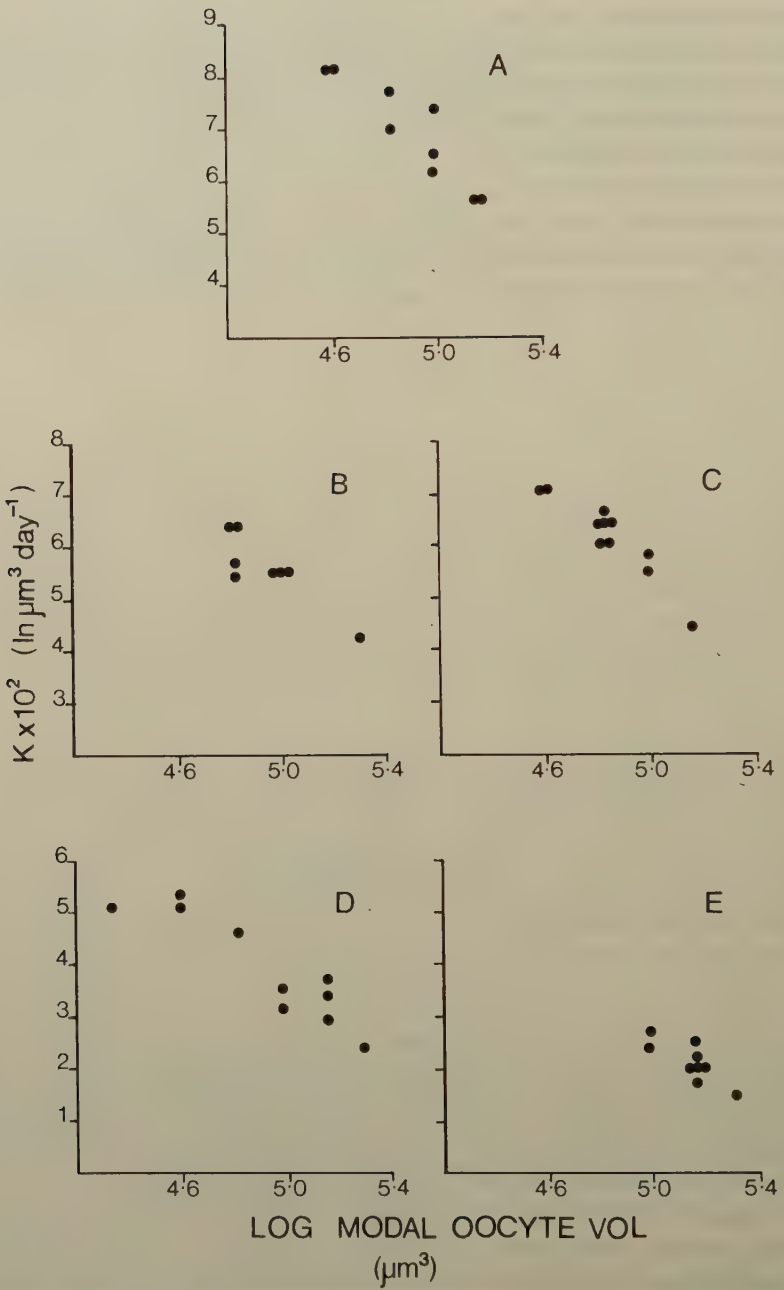

Fig. 9. Synchronization between individuals during the first wave of vitellogenic growth, under a variety of constant conditions: A, 5 °C and 16L : 8D; B, 5 °C and 13L : 11D; C, 5 °C and 12L : 12D; D, 10 °C and 12L : 12D; E, 5 °C and 12L : 12D after early entry into vitellogenesis.

induced to enter the vitellogenic phase some 2½ months ahead of normal (see p. 44). The presence of this endogenous mechanism would prevent the demonstration of an environmental synchronizing system, if it were present. Nevertheless, the influences of temperature and daylength on the first wave of vitellogenesis would tend to have a synchronizing effect. Similarly, environmental factors would tend to have the same effect over most of the other stages of gametogenesis in the two sexes, as the fundamental criterion for the operation of the hypothetical system applies equally to them. At present, however, it is not possible to demonstrate that these tendencies produce any real synchronization.

Discussion

In the Cullercoats population of *H. imbricata*, the spawning season is remarkably constant from year to year, and is apparently linked to the calendar date rather than the tidal-lunar cycle. Laboratory studies indicate that this is not the result of an environmental trigger to spawning, but rather that spawning occurs as soon as mature oocytes are present in the nephridia, providing a male is present. This implies that the control of the timing of events during gametogenesis and the synchronization of these events between individuals are the important factors in determining the spawning season.

Experimental results have shown that, although many stages in the gametogenic cycles of the two sexes are influenced by environmental conditions, these cannot be considered as directly controlling the timing of events in the reproductive cycles. It seems that an endogenous control mechanism, coupled with exogenous input in the form of information on daylength changes, provides overall control. Observations on the endogenous factor are consistent with the presence of a circannual rhythm underlying the reproductive cycle, although conclusive demonstration of such a rhythm has not yet been possible. The rôles of long-term rhythms in controlling reproduction in invertebrates has received little attention due, no doubt, to the difficulties associated with their demonstration. In the crayfish *Orconectes pellucidus inermis*, Jegla & Poulson (1970) have shown that the reproductive cycle is largely determined by a circannual rhythm, and in the polychaete *Platynereis dumerilii* Hauenschild (1960) has interpreted the persistence of the lunar rhythm of swarming activity under constant conditions as evidence for an endogenous component in the control mechanism. Recently, however, Schroeder & Hermans (1975) have put forward an alternative explanation, not involving any endogenous factor, and it is clear that the problem is compounded by the fact that the rhythmicity of swarming activity is a characteristic of the population, as each individual swarms only once before dying.

Wherever endogenous rhythms are involved, some input of exogenous information, a Zeitgeber, is necessary to synchronize the rhythms of individuals. In *Orconec-*

tes pellucidus inermis this is provided by an annual influx of fresh water, which also serves to trigger spawning (Jegla & Poulson, 1970). In *Harmothoe imbricata* there is evidence to suggest that daylength changes are performing this function. The importance of the direction of change of daylength during the first wave of vitellogenesis in determining the rate of oocyte growth in animals induced to enter this phase abnormally early may be interpreted as indicating that this is the time when the internal clock is reset. It is assumed that a similar process occurs in males, and although it is not known precisely when the Zeitgeber is effective, it is likely to be during the early processes of gametogenesis.

Synchronization of events between individuals has been shown to be under the control of an endogenous factor, at least in the case of the first wave of vitellogenesis in females, and is presumed to occur during other stages in the gametogenic cycles of the two sexes. The remarkable correlation between the direction of change of environmental factors in the field, and the direction in which they have been shown to accelerate or favour gametogenesis, raises the possibility that daylength and temperature may also have a synchronizing influence.

Recently Olive & Clark (1978) and Clark (1979) have suggested that there are two levels at which environmental factors may control reproduction. First, necessary environmental conditions for gametogenesis and/or spawning may be temporally restricted, thus broadly determining the timing of the spawning season. Secondy, specific environmental signals may be needed to trigger particular events in the gametogenic cycle, or spawning itself. As far as *H. imbricata* is concerned, it is essential that temperature falls below a critical level, between 10 and 15 °C, for gametogenesis to proceed, but it is unlikely that these necessary conditions can be determining the timing of the spawning season in nature. Specific environmental signals are effective only in the sense that the increasing daylength regime during the first wave of vitellogenesis in females is probably acting as a Zeitgeber to an endogenous clock mechanism, and it is assumed that it plays a similar rôle in males.

Acknowledgements

This work was carried out during the tenure of a Science Research Council studentship. I would like to thank Dr. P. J. W. Olive for his help and encouragement throughout, and Professor R. B. Clark, Dr. A. Brennan, and Dr. P. J. Garson for their comments on the manuscript.

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PASSIVE COLONIZATION AND ASEXUAL COLONY MULTIPLICATION IN THE MASSIVE CORAL *PORITES LUTEA* Milne Edwards & Haime

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Abstract: Heads of the reef-building coral *Porites lutea* Milne Edwards & Haime are occasionally separated or torn loose from the substratum by bioerosion or mechanical stresses. Detached heads may survive and colonize down-slope or down-stream soft bottoms, forming incipient reefs in habitats unsuitable for colonization by larval settlement. One important result of this process is reef consolidation and growth on the lagoonward edge of truncated, inter-island reef flats on atolls. In some cases, portions of the detached colonies remain attached at the original site; thus there is asexual colony multiplication.

INTRODUCTION

Despite a number of studies on the bioerosion of hermatypic coral skeletons (*e.g.*, Bertram, 1936; Otter, 1937; Hein & Risk, 1975; MacGeachy & Stearn, 1976) and storm damage to reefs (*e.g.*, Stoddart, 1963; Glynn *et al.*, 1965; Ball *et al.*, 1967; Maragos *et al.*, 1973) little information is available on the fate of reef corals that become detached from the substratum due to storms, the activities of boring organisms, or other reasons.

A few corals in both the Caribbean (*Manacina areolata*; Goreau & Goreau, 1960) and the Indo-Pacific (Fungiidae; Wells, 1954; Pichon, 1974; Maragos, 1977) are adapted to a free-living existence and have incorporated detachment into their life histories. Glynn (1974) reported on corals that form spherical heads by overgrowing algal nodules which eventually come loose and are turned over periodically by foraging fish, resulting in growth on all sides. A review of other circumrotary forms is given by Gill & Coates (1977).

Of the few reports on the fate of dislodged, reef-building corals not apparently adapted to a free-living existence, only one (Dollar, 1975) gives quantitative data on coral movement and survival. Most accounts are anecdotal or based on single observations (Connell, 1973) and nearly all deal with branching forms that are broken and scattered about during storms (Glynn *et al.*, 1965; Perkins & Enos, 1968; Maragos, 1972; Gilmore & Hall, 1976; Shinn, 1976; Randall & Eldredge, 1977; Stimson, 1978); some pieces survive, resulting in a broader distribution of the species involved.

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The site studied by Dollar (1975) on the Kona coast of Hawaii was struck by a winter storm. Post-storm measurements revealed that the peak in percentage live coral cover, including unattached corals, had shifted 10–15 m further offshore and 5–10 m deeper. For attached corals only, the peak shifted little but the percentage live cover dropped 15% at −8 m and 60% at −25 m. The maximum decrease in percentage live cover of attached and unattached corals combined was ≈17% at 10–15 m depth but at −20 m the percentage of live coral was slightly higher. The above data clearly show that many live corals or coral fragments were broken loose by the storm and transported into somewhat deeper water.

Below, I report on detachment, transport, and resultant colonization of new habitats in the massive coral, *Porites lutea* Milne Edwards & Haime. In contrast to previous studies, these measurements were not made with respect to storm activity. Storms may play a role in these events but substratum features, boring organisms, and the biology of the corals are perhaps more important.

Areas Studied

This study was conducted at the Mid-Pacific Marine Laboratory, Enewetak Atoll (11°N : 162°E). Observations and measurements on *P. lutea*, probably the most important massive coral at Enewetak (Highsmith, 1979a), were made on the intertidal reef flat between Enewetak and Sand Islands (Fig. 1) and on two coral pinnacles, North Enewetak Pinnacle and Sand Island I Pinnacle. Pinnacles are large coral formations or patch reefs that rise from the sand-covered lagoon floor to within a few meters of the water surface. There are ≈2000 pinnacles in the Enewetak lagoon (Emery *et al.*, 1954) of various size, shape and slope (Fig. 2). North Enewetak Pinnacle is ≈500 m lagoonward of the north end of Enewetak Island and Sand Island I is a similar distance directly lagoonward of Sand Island.

Results and Discussion

LAGOON PINNACLES

P. lutea heads are often abundant in level or gently sloping sandy areas adjoining the bases of pinnacles. Preliminary examination of these heads revealed that many of them were not attached to a substratum but lying free on the sand (Fig. 3). I, therefore, counted and measured attached and loose *P. lutea* heads as a function of depth from the top of each pinnacle down one side and out onto the adjacent sand plain at −40 ft (−12.3 m). More of the *P. lutea* heads on sand are loose than on pinnacles (Fig. 4; 2 × 2 contingency table, $\chi^2 = 87.4$; $P < 0.001$). Also, loose *P. lutea* heads are significantly larger than attached heads (Mann–Whitney *U*-test, $P < 0.001$). The mean diameters of loose heads were 20 cm and 22 cm at the two pinnacles and the mean diameter of attached heads was 13.5 cm at both locations.

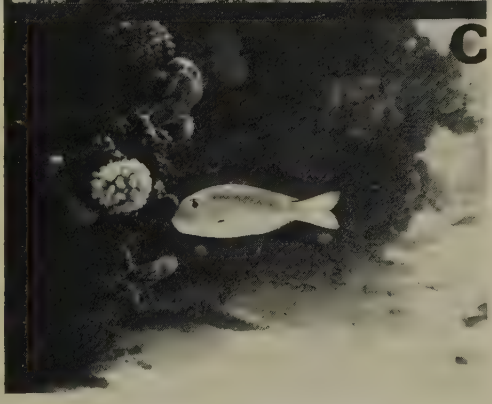

Fig. 1. Windward margin of Enewetak Atoll showing Enewetak Island (a), Sand Island (b), and reef consolidation zone (c).

Fig. 2. Enewetak Atoll: A, lagoon pinnacle with steep sides, depth ≈13 m; B, upper slope of a lagoon pinnacle, depth ≈8 m; C, side of a patch reef, depth ≈5 m; detached corals on reefs are likely to move downslope.

Fig. 3. Loose head at Sand Island Pinnacle I (background): scar (arrow) indicates that this head was previously attached to the pinnacle and is now upside down relative to when it was attached.

Since coral planulae evidently cannot settle successfully on soft bottoms (Marion, 1883; Vaughn, 1909; Stephenson, 1931; Hada, 1932; Motoda, 1939; Hiatt, 1958; Harrigan, 1972), the loose *P. lutea* heads on the sand were undoubtedly originally attached to the pinnacles. In fact, scars indicating former attachment are still present on some loose heads (Fig. 3). Coral heads that become detached from the substratum on pinnacles are likely to move down-slope (Fig. 2) to relatively level areas.

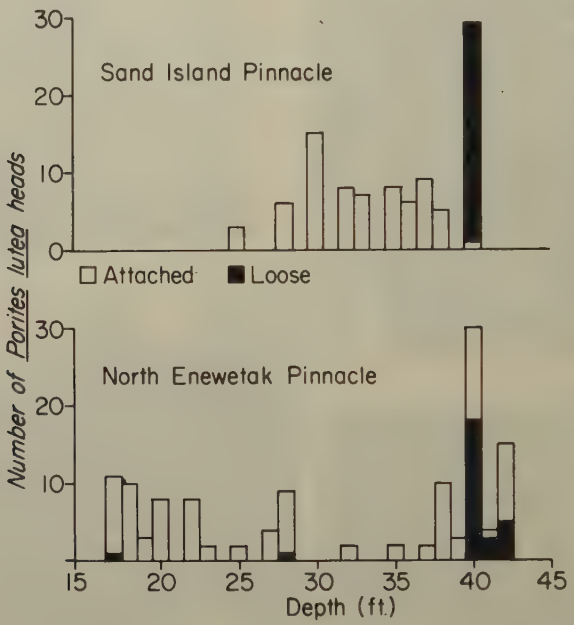

Fig. 4. Depth distribution for loose and attached *Porites lutea* colonies on two lagoon pinnacles at Enewetak Atoll: sand substratum begins at −40 ft (−12.3 m).

Relative to other massive species, *P. lutea* is adept at maintaining live tissue over its base, thus protecting the base from boring organisms and permitting enlargement of the base commensurate with colony growth (Highsmith, 1979b). *P. lutea* does not, however, maintain live tissue more than 2–3 cm under a shaded lip or overhang on its skeleton. If the base of a head is killed or temporarily buried for some time, or is situated on a substratum of limited area, an overhang may form at the margin of live tissue. These heads will have weak, unprotected bases and are presumably the source of most loose heads. Strongly attached heads may also be knocked loose during storms (Highsmith, 1979b).

The size difference between loose and attached heads suggests that either larger heads are more likely to come loose or that larger heads are more likely to survive if they become detached and tumble out onto the sand, or both. The attached heads on sand (−40 ft) at North Enewetak Pinnacle (Fig. 4) were growing on large pieces

of partially buried rubble. These could have originated from planular settlement or have fallen from the pinnacle along with the piece of substratum to which they were attached. The colonization of sand bottoms by *P. lutea* may initiate a

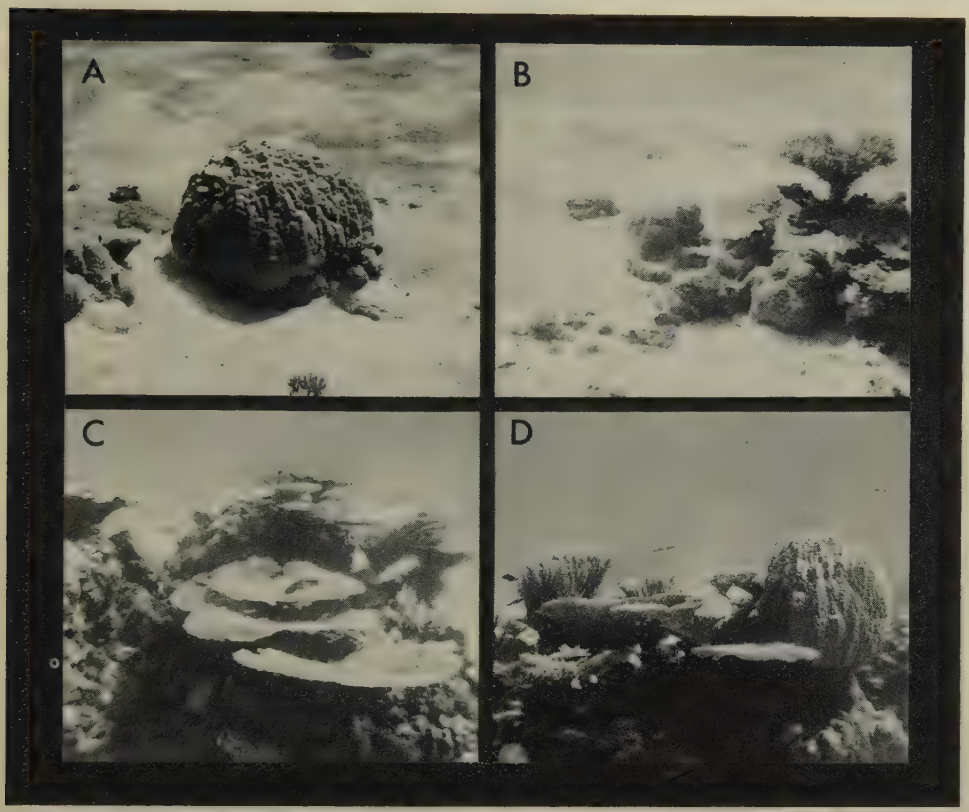

Fig. 5. Possible stages, A to D, in patch reef formation: A, *Porites lutea* head ≈0.7 m diam. on sand bottom with a few pieces of rubble and two coral recruits around margin; B, small patch reef composed of accumulated rubble, corals, and coral recruits; C, larger patch reef with vertical development due to growth of corals; D, well developed patch reef; note large *P. lutea* with limited substratum for basal growth, suggesting that it may eventually topple on to the sand and initiate a new cycle of reef formation.

sequence of events (Fig. 5) leading to patch reef formation. Rubble or other heads may come to rest against colonists (Fig. 5A), increasing the probability of trapping more material (Fig. 5B) and providing a substratum for settlement by coral larvae (Fig. 5C,D).

REEF FLAT

P. lutea occurs in the form of microatolls (Fig. 6) on intertidal reef flats in the Marshall Islands (Wells, 1954) and elsewhere (Scoffin & Stoddart, 1978). The tops

of these massive corals are killed by prolonged exposure during seasonally low tides. The coral tissue around the periphery of the head survives and continues to grow radially outward resulting in the characteristic microatoll form (Scoffin & Stoddart, 1978; Highsmith, 1979b). At Enewetak, such microatolls occur on reef flats between islets where water flow is unidirectional from ocean to lagoon. The evidence suggests that some microatolls become detached from the reef flat and are moved by currents to the lagoon.

Fig. 6. *Porites lutea* microatolls on reef flat.

P. lutea microatolls are often rather weakly attached to the substratum for two reasons. First, as mentioned above, *P. lutea* does not maintain live tissue more than 2–3 cm under shaded skeletal projections (Highsmith, 1979b). When, therefore, an overhang develops around the edge of the microatoll, the live coral tends to lose contact with the substratum so that only a fraction of the coral's lower surface is actually attached to the reef limestone. Secondly, the basal attachments of the microatolls are often weakened by boring organisms, especially by clionid sponges and, unlike *P. lutea* on lagoon pinnacles, by the burrowing sea urchin, *Echinometra mathaei* (Blainville) (Fig. 7).

Along the lagoon edge of the reef flat is a zone of live coral, mostly *Porites lutea*, and much of the coral in this zone appears to have originated as microatolls (Fig. 8). Microatolls plus rubble transported to this zone by currents are consolidated into solid reef structure by continued growth of *P. lutea* (Fig. 9). It is estimated that

the volume of calcareous material in this consolidation zone (Fig. 10) is about 1200 m^3 with a weight of about 1.75×10^6 kg.

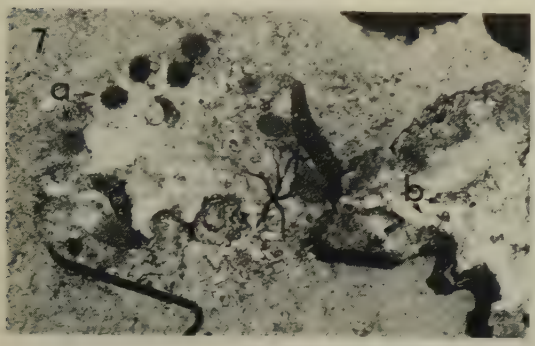

Fig. 7. Attachment site and underside of a microatoll: the burrowing sea urchin, *Echinometra mathaei* (a) and clionid sponges (b) weaken microatoll attachment to the reef.

Fig. 8. Live *Porites lutea* in the consolidation zone: note abundance of circular microatolls (arrows); upper microatoll in B is upside down, revealing *Echinometra mathaei* burrows.

Fig. 9. Edge of consolidation zone at location indicated by asterisk in Fig. 10: note vertical relief which increases towards the lagoon; waves mark lagoonward edge of the consolidation zone.

The time required for development of the consolidation zone is difficult to estimate. Current patterns suggest that unattached microatolls on about one half of the ≈400-m wide reef flat (Prof. Pap. U.S. Geol. Surv. No. 260, Chart 5) would be transported to the consolidation zone. The mean diameter of 136 microatolls along two 2-m wide transects from the edge of the consolidation zone to the algal ridge was 72 cm (median 61 cm; range 24–231 cm). Using an average microatoll thickness of 9 cm and a skeletal density of 1.4 $g \cdot cm^{-3}$ (Highsmith, 1979a, b) for *P.*

lutea, gives a mean microatoll weight of ≈50 kg. Based on this microatoll abundance, size, and a growth in radius of ≈1 cm/yr (Highsmith, 1979a), about 185 yr would be required to produce the amount of calcareous material in the consolidation zone, assuming no other source of calcium carbonate. Half as long would be required if microatolls from the entire reef flat contribute to build-up of the consolidation zone. If only microatolls directly seaward of the consolidation zone are considered, ≈400 yr would be required. Factors such as transport of rubble and other corals to the zone, growth of corals in the zone, and possibly larval recruitment would shorten the development period.

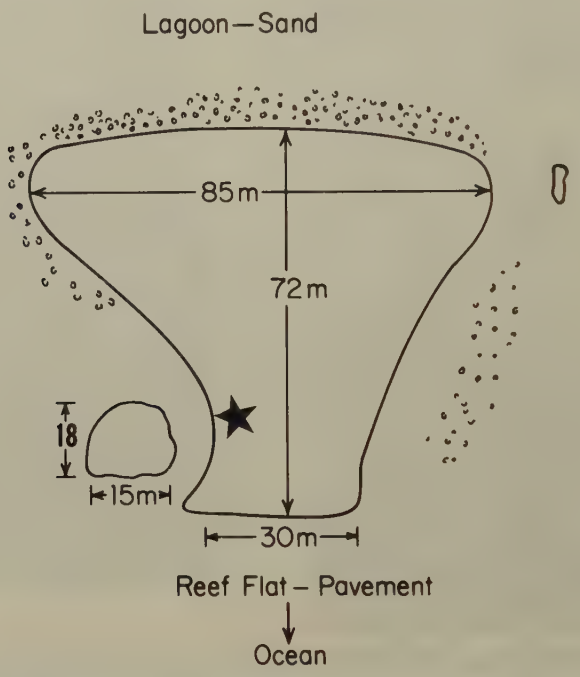

Fig. 10. Sketch of the consolidation zone showing approximate dimensions: see Fig. 1 for location on reef.

It is suggested by the size distribution data and growth rate of *P. lutea* (see above) that microatolls typically have a residence time on the reef flat of 30–40 yr. Even allowing for slower *P. lutea* growth rates on pinnacles due to greater water depth (Highsmith, 1979a), the microatolls appear to be ≈2–3 times older on the average than attached heads on pinnacles. Thus, recruitment and/or mortality rates are different on the reef flat than on lagoon reefs.

ASEXUAL COLONY MULTIPLICATION

Living fragments of *P. lutea* often remain attached to the substratum where a microatoll was previously situated (Fig. 11). This is because live coral tissue on the periphery of microatolls ocasionally makes contact with the substratum, attaches,

Fig. 11. Small colonies of *Porites lutea* (arrows) that were part of a single microatoll carried away by currents: see text.

and grows out onto it. When the microatoll becomes detached, these peripheral encrustments break off from the colony and remain attached to the reef, resulting in asexual colony multiplication. Thus, an individual *P. lutea* may colonize a new habitat (sand bottom), remain in its original location, and reproduce asexually all at the same time. I suggest that most microatolls on the reef flat at Enewetak originate from previous microatolls in the manner outlined above. Similar events may occur on pinnacles but are not as easy to detect as on reef flats where colonies are situated on a more homogeneous substratum and have a characteristic shape.

General Discussion

The results raise the following questions: is detachment from the substratum adaptive in *P. lutea*? In addition to the possibility of asexual reproduction, are there advantages of detachment that outweigh the disadvantages? For corals in general, how common is this mode of reproduction and/or colonization of new habitats?

Among the more likely disadvantages are: physical damage while moving, burial, scouring, instability, and landing in a less favorable location. Physical damage will depend upon distance travelled, velocity and number of obstructions encountered. If a coral comes to rest on sand, burial and/or scouring may occur as the sand is moved about by currents. Unattached heads may be unstable and moved again during subsequent storms. At one site, I counted 26 loose heads. Nine months later at the same place, twice the effort resulted in finding only 12 loose heads. Whenever a coral head is moved to a new location there is a finite risk that the new site will be less favorable than the original. On pinnacles, unattached corals tend to move downslope into deeper water where there is less light and possibly a greater sediment load. If the new depth were great enough, depending upon the species, the coral might experience reduced growth rates (Highsmith, 1979a) or even perish.

On the other hand, by moving from the reef frame to sand, *P. lutea* may avoid competitors (Highsmith, 1979b) or reef-bound predators such as parrotfish (Scaridae) or *Acanthaster*, although the latter prefers other coral species to *Porites lutea* (unpubl. obs.). In addition, large colonies are able to colonize a new, and in many cases quite suitable (Cary, 1931; Fig. 5A) habitat (sand) that planulae are unable to colonize. Water depth at the lagoon edge, for example, is greater than on the reef flat so that *P. lutea* colonies can potentially grow much larger (Fig. 8A) before the tops are killed by low tides (Fig. 6).

The importance to corals of enlarging their base commensurate with colony growth and protecting it from destruction by boring organisms (Highsmith, 1979b) leads to the prediction that corals unable to survive detachment should invest more energy in maintaining live tissue over their base than those that can survive. Because branching corals are severely weakened if colonized by boring organisms there should be strong selection for branching forms to keep live tissue over their lower branches and base, at least until the branches or colonies are large enough to survive detachment (Gilmore & Hall, 1976; Shinn, 1976; Birkeland *et al.*, 1979).

The events described or hypothesized above may be rather common among corals. For example, patches 10–13 m across of the deep-water, ahermatypic coral *Lophelia pertusa* may develop from a single colony (Wilson, 1979). Lower parts of the skeleton not protected by live tissue are weakened by clionid sponges, resulting in collapse of branches onto the surrounding sediment. There is outward development of the patch because some branches survive and those that do not provide a substratum for lateral colony growth or planular settlement. In hermatypic corals, many Caribbean *Montastrea annularis* colonies occur as plate, columnar, hemispheroidal, or dome-shaped growth forms (Goreau & Hartman, 1963; Scatterday, 1974; Smith & Tyler, 1975). The plate and columnar forms are not a very stable shape (Highsmith, 1979b) and the unprotected bases are extensively bioeroded (Goreau & Hartman, 1963; Scatterday, 1974; MacGeachy & Stearn, 1976), yet both forms tend to survive detachment (Lewis, 1960; Goreau & Hartman, 1963; Scatterday, 1974). In the columnar growth forms, there may be asexual reproduction in

instances where the growth surface of a single, hemispheroidal head becomes divided, resulting in the production of several to many columns (Lewis, 1960; Scatterday, 1974, 1977) that eventually detach. The hollow, dome-shaped *M. annularis* colonies eventually collapse, breaking into several independent colonies that contribute to patch reef formation (Smith & Tyler, 1975). Similar events result in the break-up of *Pavona clavus* domes in the Gulf of Chiriqui, Panama (unpubl. obs.). Among branching hermatypes, *Pocillopora damicornis* in the eastern Pacific (Glynn *et al.*, 1972), *Acropora aspera* in Guam (Birkeland *et al.*, 1979), and *Acropora cervicornis* in Florida (Gilmore & Hall, 1976) frequently reproduce and gain broader distribution by breakage of branches or clusters of branches from parent colonies.

Since the majority of coral reefs occur in regions of hurricane activity (Stoddart, 1971), the frequency of severe storms may be an important factor in coral growth characteristics and reproductive patterns. In the Caribbean where long-term meteorological data are available, there were 286 hurricanes ($\bar{x}$ = 4.5/yr) and 212 tropical storms of less severity ($\bar{x}$ = 3.4/yr) between 1900 and 1962 (Glynn *et al.*, 1965). Stoddart (1963) estimates that hurricanes strike Belize reefs every 10 yr and Ball *et al.* (1967) calculate that over the last 10 000 yr any particular Florida reef has been affected by hurricanes once every 6 yr on the average, i.e. a total of over 1650 hurricanes. Clearly, storms have great potential as a selective agent in the evolution of corals capable of surviving detachment and possibly capitalizing on storms to reproduce by colony fragmentation.

The detachment and re-distribution of hermatypic corals is an important and generally overlooked process on reefs that leads to the colonization of otherwise inaccessible habitats, patch reef development, reef extension, and asexual colony multiplication.

Acknowledgements

I thank Drs. S. Smith and E. S. Reese for use of facilities at the Mid-Pacific Marine Laboratory. I am grateful to J. Lamberson, P. Lamberson, and especially A. Riggs for field assistance. Drs. A. J. Kohn and R. T. Paine read the manuscript. The work was supported by NSF grant No. OCE 76–21271, the Mid-Pacific Marine Laboratory, and D. D. Highsmith.

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J. exp. mar. Biol. Ecol., 1980, Vol. 47, pp. 69–75
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AN ELECTROFISHING APPARATUS FOR SAMPLING SUBLITTORAL BENTHIC MARINE HABITATS

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Abstract: An electrofishing apparatus using low voltage pulsed DC current which stimulates rock (spiny) lobsters *Panulirus cygnus* George to move out of shelter and an AC current to stun them to allow capture, is described.

The unit is powered from a portable 250 V, 2.5 KVA alternator. Pulses of $\approx 25\ V \cdot m^{-1}$ were sufficient to induce movements of the rock lobsters. A 50 Hz AC field of $\approx 10\ V \cdot m^{-1}$ sustained for ≈ 5 min was found to immobilize the rock lobsters for several minutes. A special feature is that it incorporates a capacitative discharge circuit with series inductor to produce the required pulse shape and to put the maximum energy into the water. Heat dissipation problems in the capacitative charging circuit are overcome by replacing the charging resistor by an inductor.

The electrofishing apparatus allowed capture of a greater size range of rock lobsters and with greater speed than by baited pot techniques. It also permitted capture of non-feeding, post-moult individuals. Crabs, shrimps and fish were caught incidentally during testing. After being stunned by the apparatus, the animals could be collected by hand by divers. Maximum safety features are incorporated in the design.

INTRODUCTION

Sampling juvenile rock (spiny) lobsters in their first year after settlement from the plankton has always been a problem. At this phase in the life cycle the young rock lobsters hide in weed beds and among mangrove roots, etc. (Kanciruk, in press). In the case of *Panulirus cygnus*, endemic to Western Australia, laboratory studies suggest that these young rock lobsters are light sensitive and are probably not gregarious (Phillips *et al.*, 1977). Normal collecting techniques such as baited traps and diving have been unsuccessful in catching more than isolated specimens, because of both the difficulty of penetrating the weed beds, etc. and the behaviour of the animals.

Sweat (1968) attempted the capture of young *Panulirus argus* using several methods including artificial shelters, push nets, a roller-frame trawl and even a vacuum dredge, but caught only a total of two lobsters by all these methods. Greater success was reported by Engle (1977) who captured small numbers of young *Panulirus inter-*

ruptus by hand in surf grass in California using SCUBA. Some young *P. cygnus* have been caught using similar techniques, at night, in Western Australia (Joll, pers. comm.). Hand capture using diving, however, introduces the problem of behavioural responses both of the lobsters and the diver.

Although electrofishing has been used in freshwater studies for many years its use in marine environments is relatively new. The higher conductivity of sea water presents more engineering problems in marine electrofishing than in fresh water and its use in the marine environment has been principally restricted to commercial non-crustacean fishing (Vibert, 1967). Commercial electrofishing trawl systems have, however, been developed for *Nephrops norvegicus* by Stewart (1974) and for *Homarus americanus* by Saila & Williams (1972) using relatively low voltages.

The system developed in the present study was designed for sampling sublittoral benthic marine habitats. The intention was to use electrical shocks to stimulate young *Panulirus cygnus* George to move out of the dense cover in which they shelter, i.e. the base of the weed growths, and then stun them, to allow collection by a diver. Two other considerations were, that the electrical apparatus had to operate from a portable power source and that the design and method of operation ensure a maximum level of operator safety. The electrofishing apparatus developed is described and results of field testing are presented.

The Apparatus

The apparatus consists of two parts: a pulse generator which produced a sequence of short high-energy pulses to initially move the rock lobsters out of cover and a transformer to produce a 50 Hz AC stunning field. It was powered by a petrol driven 250 V, 2.5 KVA portable alternator and could be easily fitted into a small workboat.

The high energy pulses were produced by the capacitive discharge circuit shown

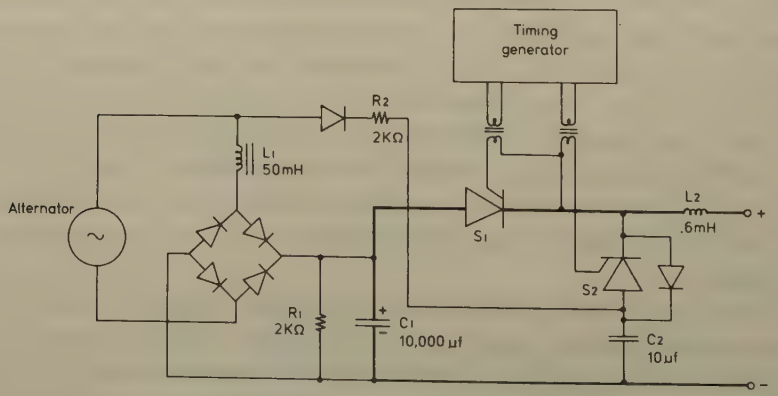

Fig. 1. Diagram of pulse generator circuit.

in Fig. 1. It differs from the usual capacitive discharge circuit in a number of ways. The use of inductor L_2 in series with the electrodes slows down the discharge to produce a rounded pulse shape found from tank tests to be more effective in inducing rock lobster movement (see p. 73). This inductor was adjusted to give not quite critical damping to produce the pulse shape and to get as much energy as possible into the water. The use of a series inductor made necessary the unusual commutating circuit comprising S_2 and C_2.

The size of the main storage capacitor C_1 used was the largest which could be charged up in the time between pulses. A second inductor (L_1) has been used in the main charging circuit instead of a resistor to limit the short circuit charging current to the maximum that can be supplied by the alternator in the short charging period. The use of an inductor here eliminates a major source of power loss and, therefore, heat generation.

The AC stunning field was obtained using a step-down transformer which gave the required high current as well as voltage isolation for safety. The transformer secondary was provided with taps which could be switched to give maximum field strength without overloading the alternator.

The overall configuration of the electrical apparatus is shown in Fig. 2. Connec-

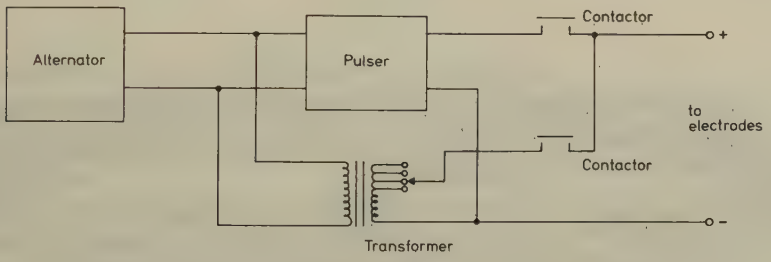

Fig. 2. Overall configuration of the electrofishing apparatus.

tion to the electrodes was by contactors which automatically isolated the electrodes when the equipment was not operating. Additional relays, not shown in the diagram, were used to also isolate the SCR firing circuits for maximum safety. All equipment was thoroughly water proofed and the electrodes were attached by screwed connectors to prevent accidental removal.

Field tests of the apparatus were made using two types of free standing electrodes, each designed for sampling a different habitat.

(1) A circular array with an outer (−ve) ring 4 m in diameter and an inner (+ve) ring 0.5 m in diameter (Fig. 3a) constructed from 2-cm diameter stainless steel tubing with heavy duty welding cable to connect to the apparatus. All tubes were 30 cm high. As illustrated this apparatus was designed for use in weed beds. With the circular electrodes a voltage of ≈ 40 V could be used to give, after

allowing for electrode loss, a field strength of $\approx 10\ V \cdot m^{-1}$ near the outer ring and increasing towards the inner ring.

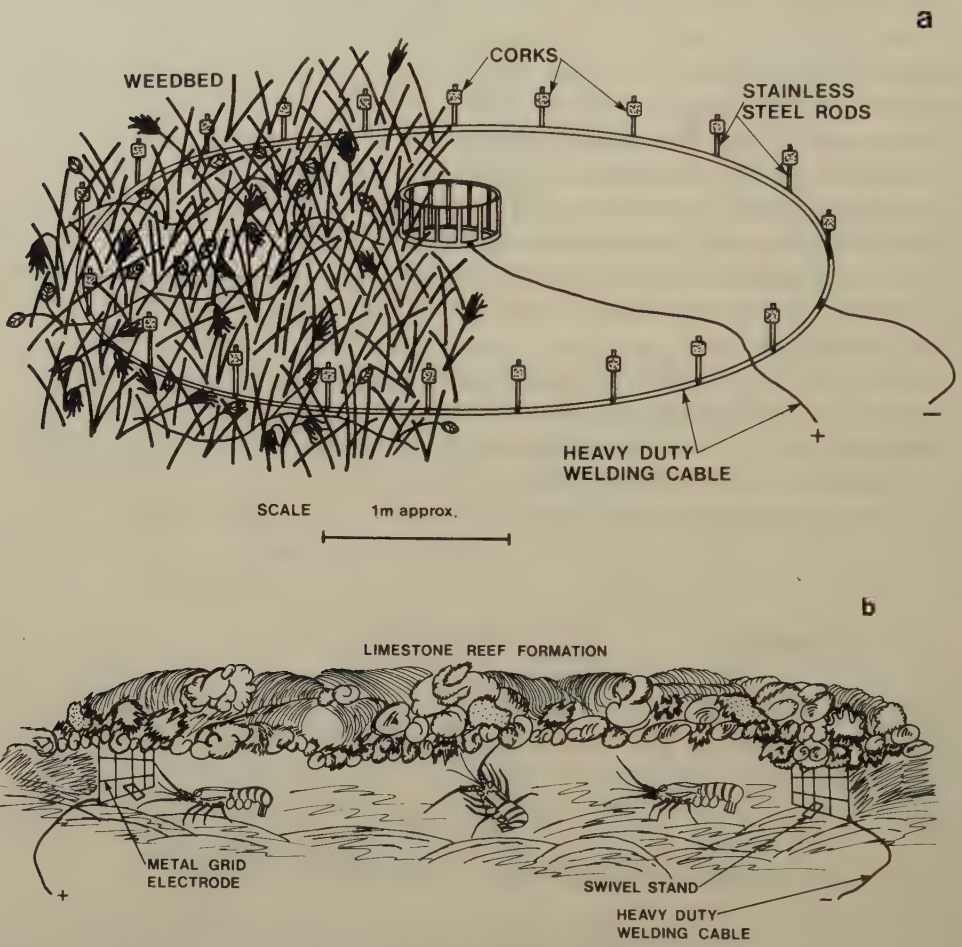

Fig. 3. a, circular electrode array designed for use in weed beds; b, grid electrodes, designed for use along reef ledges.

(2) Two metal grids 0.4 × 0.2 m were constructed from 2-cm steel (Fig. 3b) tubing. These electrodes were designed for use along reef ledges. With the two grid electrodes set ≈3 m apart, the voltage could be increased to ≈60 to 80 V depending on the size and overhang of the reef ledge.

Methods

Before constructing the apparatus a series of tank tests was carried out in the laboratory using narrow PVC tanks and flat plate electrodes to observe the response of shocked lobsters under simulated field conditions. The effect of different pulse shapes, number and frequency of pulses was tested on the size range of lobsters it was proposed to capture. The lobsters used in the tests had been raised in the laboratory from puerulus stage animals caught using collectors of artificial seaweed (Phillips, 1972).

Field tests of the apparatus were carried out over 2 days using both sets of electrodes at Seven Mile Beach, ≈ 300 km north of Perth in Western Australia, an area known for high puerulus settlement (Phillips, 1972).

Maximum safety during operations was achieved by turning off the alternator before allowing the divers to enter the electrode field to collect specimens. However, using the recommendations of Stewart & Cameron (1947) for underwater observations of an electrode array on the sea bed, it was possible to observe the effects of electrofishing apparatus on the lobsters under the reef ledges.

Results

The following results were obtained from the tank tests.

(1) Pulses at field strengths of $\approx 25\ V \cdot m^{-1}$ were found sufficient to induce muscular contraction of the abdomen of the rock lobster and hence induce violent movement.

(2) The rock lobsters were sensitive to the polarity of the field and either faced or propelled themselved towards the +ve electrode.

(3) The most effective pulse shape tested was a rounded exponential similar to the pulse which Meyer-Waarden (1957) calls "unperiodic".

(4) The most effective pulse pattern to induce movement was two pulses of about 5 ms duration 200 ms apart, followed by two more such pulses after an interval of 2 s.

(5) A 50 Hz AC field of $\approx 10\ V \cdot m^{-1}$ sustained for ≈ 5 min was found to immobilize the rock lobsters for several minutes. The sustained current prevented the operation of the baler organ used in respiration. Times up to 15 min were tested. All animals recovered, although some exposed to long times took up to 20 min to revive and become mobile.

(6) With extreme field strengths of more than $2000\ V \cdot m^{-1}$ it was possible to cause leg losses, but no rock lobsters were ever killed.

(7) Laboratory measurements showed that the electric current passed through seagrass beds and, where rocks or reef ledges were present, concentrated along the inner edges of such objects.

In the field underwater observations showed that the effect of the current was instantaneous, with those rock lobsters nearest to the electrode grids being the first to be overcome. To capture those animals visible within the field of the grid electrodes under the ledges it was necessary only to apply the AC field without the use of the initial DC pulse series designed to force animals out of shelter.

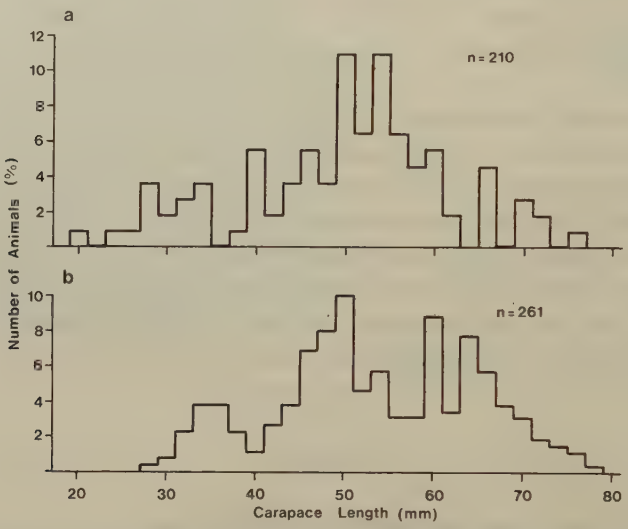

Fig. 4. a, size range of the juvenile *Panulirus cygnus* caught at Seven Mile Beach in Western Australia during 2 days of testing of the electrofishing apparatus; b, size range of the juvenile *Panulirus cygnus* caught at Seven Mile Beach by baited traps.

The size range of the juvenile *P. cygnus* captured during the 2 days of field tests is shown in Fig. 4a. This may be compared to the size range of *P. cygnus* caught by baited traps in this area over a full year by Chittleborough (1974) shown in Fig. 4b. The apparatus was successful during these tests in capturing crabs, shrimps and several species of fish, which happened to be within the field of the electrodes.

Discussion

The rock lobsters caught during the field tests and using the electrical apparatus include several which were smaller than those caught with the baited traps, while the upper size limit was similar. This is of particular interest because the data of Chittleborough (1974) included the catches of special traps with semicircular screens placed in front of the entrances designed to catch very small rock lobsters.

The carapace size of newly settled rock lobsters is ≈ 8 mm (Phillips, 1972). Fig. 4a indicates that rock lobsters of carapace size 8–20 mm were not captured by the electrofishing apparatus. The tests made were by no means exhaustive, however, and

modifications and different positioning of the electrodes in the reef area may permit capture of animals in this size range. For example, "honeycombed" reef structures in the area were not accessible with the present electrodes but may contain newly settled rock lobsters.

Capture during these tests of the full size range of rock lobsters previously caught by baited traps suggests the potential use of the electrofishing apparatus to obtain samples of rock lobsters for such studies as nutrition where the use of a baited trap is inappropriate. Of special importance was the capture of a number of rock lobsters with the exoskeleton in the "soft" immediate post moult condition. Animals in this moult stage do not feed (Phillips, 1975) and are therefore not sampled by baited traps nor because they hide are they caught by divers.

For rock lobster sampling the apparatus is at least as effective and more rapid than baited pot techniques with the advantage of also capturing non-feeding individuals. That the electrofishing apparatus was also useful for collection of other types of marine animals suggests its possible use in experimental ecological studies for removal sampling of benthic macrofauna from reef areas. The apparatus developed is portable, relatively inexpensive to construct and incorporates maximum safety features in the design.

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J. exp. mar. Biol. Ecol., 1980, Vol. 47, pp. 77–87
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SEDIMENT REJECTION BY REEF CORALS: THE ROLES OF BEHAVIOR AND MORPHOLOGY IN *MONTASTREA CAVERNOSA* (Linnaeus)

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Abstract: The sediment rejection abilities of colonies of *Montastrea cavernosa* (Linnaeus) were monitored in a series of field and laboratory experiments. Under conditions of natural sediment deposition, colonies were capable of removing virtually all sediment from their surfaces. Equivalent sections of dead coral skeleton accumulated as much as 345 mg sediment $\cdot$ 25 $cm^{-2} \cdot day^{-1}$. Laboratory experiments show the sediment rejection process to consist of passive and active phases. Passive movement of sediment off colonies occurs at the time of initial sediment impact. Fine grain size, tall polyps, and convex colonies all enhance passive removal. Active removal involves the behavioral responses of the colony and is independent of sediment type or colony morphology. The presence of appropriate morphologies can increase the proportion of sediment passively removed and reduce the amount of work a colony must perform to keep its surface clear of sediment.

INTRODUCTION

The effects of sedimentation and sediment resuspension on reef corals have always been recognized as important factors controlling coral abundance and distribution (c.f. Marshall & Orr, 1931). In the Caribbean and West Atlantic, several authors have recognized a coral fauna characteristic of areas with high rates of sedimentation and resuspension (Lewis, 1960; Laborel, 1969; Loya, 1976). Corals like *Montastrea cavernosa*, *Diploria strigosa* and *Siderastrea siderea* which dominate this fauna are frequently of secondary importance on other reefs and have all been characterized as effective sediment clearers by Bak & Elgershuzien (1976). *Montastrea cavernosa* (Linnaeus), a common species on many Caribbean reefs, is an important member of this sediment resistant fauna. On most reefs it is dominated by its congener *M. annularis*, which ranks above *M. cavernosa* in aggressive ability (Lang, 1973). However, *M. cavernosa* supersedes *M. annularis* in environments dominated by rapid sediment accumulation (Loya, 1976).

The abundance of *M. cavernosa* on reefs with rapid sediment accumulation suggests the importance of sediment removal in the ecology of *M. cavernosa*. Examination of sediment rejection by *M. cavernosa* is also of interest due to this species' exceptional intraspecific variability. In Panama, two morphs of *M. cavernosa* have been distinguished on the basis of morphology and behavior (Lehman & Porter,

1973; Lasker, 1976, 1978). The diurnal morph of *M. cavernosa* is characterized by continuous polyp expansion and small polyps (≈5 mm diameter) which are closely spaced and have short calices. The nocturnal morph has polyps which are large (>6 mm), distantly spaced, have tall calices and expand only at night. The variability exhibited by *M. cavernosa* facilitates an analysis of the roles of morphology and behavior in sediment rejection.

EFFECTS OF SEDIMENT ON CORALS

Sediment can inhibit coral growth in three general ways. Sediment-covered surfaces may prevent planula settlement, the presence of sediment in the water column or on the colony surface may slow growth, and rapid sediment deposition may lead to burial and death.

The role of substratum availability in controlling coral distribution is undoubtedly important. Birkeland (1977) found survival of corals on the undersides of Plexiglas settling plates to be greater than on the top surface. This was interpreted as an effect of the greater algal cover and sediment deposition on the upper surfaces.

Reduced growth in areas of resuspension and high turbidity have been observed by several authors. Dodge *et al.* (1974) related the lower growth rates of lagoonal (as opposed to fore reef) corals in Discovery Bay, Jamaica to higher sediment resuspension rates. In Bermuda, Dodge & Vaisnys (1977) correlated periods of reduced growth with extensive harbor dredging. Loya (1976) examined two reefs in Puerto Rico and found that mean colony size was greatest on the reef with the lower turbidity.

The basis for the reduced growth rates which these authors have observed is not yet clear. Turbid, sediment-laden waters reduce the amount of available light, which of course will reduce photosynthesis. At Galeta Island, Panama, for instance, sediment resuspension occurring during the dry season reduces light (PAR) availability at 9 m from 20% of surface light to <3% (Lasker, unpublished data).

Reduced growth may also result from direct metabolic costs associated with sediment cover. Sediment on the colony surface reduces light availability and may inhibit exchange of oxygen, carbon dioxide, and metabolites. Furthermore, the hydrostatic pumping, ciliary action, and mucus production which are employed in the sediment rejection process (Hubbard & Pocock, 1972) all require an expenditure of energy or resources. This diversion of resources from other functions may account for reduced growth rates.

The most severe effect of sedimentation and sediment resuspension is the death of a colony or some portion of its tissue. When sediment loading exceeds the rate of removal, a sediment layer builds up which may become anoxic and kill the underlying tissue. This phenomenon is frequently observed on the concave surfaces of colonies or along the edges of colonies resting on sand substratum.

Reduced growth and the possibility of tissue death both suggest that the rapid

removal of sediment from a colony surface is advantageous to the colony. A countering influence is the cost of removing sediment which may increase with the speed of sediment rejection. Morphologic variation can result in gradients in sediment rejection capabilities which may not involve different energetic investment. Hubbard & Pocock (1972) observed systematic differences in sediment rejection in corals with differing polyp geometry. Bak & Elgershuzien (1976), however, found that behavioral and other differences supersede morphologic characteristics. Bak & Elgershuzien portray the sediment rejection process as specific to both the coral species and the sediment type. This report examines the roles of morphology and behavior in controlling rates of sediment rejection by colonies of *M. cavernosa*.

Methods

Field and laboratory experiments were conducted at the facilities of the Smithsonian Tropical Research Institute (S.T.R.I.) in the Republic of Panama. Field observations were made at Galeta Island (9°24′N; 78°52′W) and at Nalu Nega in the Islas' San Blas (9°33′N; 78°54′W). Experiments were conducted at the S.T.R.I. Galeta Marine Laboratory.

Field Observations

Sediment clearing in nature was assessed by measuring the amounts of sediment accumulating on 25-cm^2 sections of both living and dead coral. Samples taken from dead coral (henceforth called corallum) provide an estimate of the amount of sediment which could potentially accumulate on living tissue. Differences in sediment accumulation between living-tissue and corallum samples are, therefore, estimates of the amount of sediment removed by the colony.

Unlike estimates based on sediment trap data, this index of sedimentation is coupled to the abiotic factors which normally affect sediment accumulation on living tissue. The index is, however, subject to several biasing factors which may overestimate the sediment load which would naturally come to rest on a living colony. The corallum presents a much rougher surface than living tissues and sediment is more apt to lodge in the septa or on the rough coenosteum than would occur on a living coral. Filamentous algae growing on dead substratum can also increase retention; however, in this study all coralla used for measuring sediment deposition were scrubbed clean of algae on the first day of the experiments.

At Galeta, samples were collected by carefully suctioning all sediment inside a 25-cm^2 grid with a 50 cm^3 syringe. Large accumulations sometimes occurred at Galeta and several syringes were commonly used for each sample. The *M. cavernosa* colonies sampled at Galeta all had extensive areas of dead skeleton and these were used for the corallum samples. The areas used were always located within 1 m of the living colony and were chosen to have the same orientation as the living tissue. Corallum

and living tissue areas were cleared of all sediment 24 h prior to the first sample and were cleared again immediately after samples were taken. Five colonies (living tissue and corallum) were sampled on each of 5 days.

Samples collected from living tissue in the San Blas were collected as described above. Colonies at this site, however, rarely had dead areas suitable for sampling. Instead 25-cm^2 blocks of *M. cavernosa* were placed either adjacent to the sampled colonies or on the colony itself. In no cases were the blocks placed on the sand substratum. These blocks were removed daily and the accumulated sediment carefully washed off. Blocks were kept in plastic bags during removal to prevent the loss of collected sediment. Ten colonies and blocks were sampled on each of 10 days.

The sediment samples collected at Galeta and in the San Blas were drained of sea water by filtering through Millipore glass fiber prefilters and the collected sediment dried and weighed.

LABORATORY EXPERIMENTS

Experiments were conducted on 21 small (<15 cm diameter) *M. cavernosa* colonies. The colonies were kept in water tables in shaded sunlight with continuously running sea water. All colonies exhibited normal behavior throughout the period of the experiments. Based on their expansion behavior 11 colonies were classified as diurnal and 9 as nocturnal. Experiments measured rates at which artificially deposited sediment was removed from colonies. Sediment used in the experiments was wet sieved from samples collected on the reef at Galeta Island. Only coarse (500–1000 μm) and fine (60–250 μm) fractions were used. The experiments were conducted in flowing sea water and were started at 1100. Ten to 20 colonies were tested in each experiment and each colony was tested a total of 10 times (5 with coarse sediment, 5 with fine).

At the start of the experiment 20 cm^3 of either coarse or fine sediment was deposited on each of the colonies. The sediment was evenly spread over a 100-cm^2 area which included the entire test coral. Sediment loading as determined by the amount of sediment landing in a 66.4-cm^2 Petri dish was 74.4 mg dry weight $\cdot$ cm^{-2} of the coarse sediment and 18.5 mg $\cdot$ cm^{-2} fine sediment. A photograph of each colony was taken at the start of the experiment and again after 30, 60, 120, 240, 360, and 480 min. Sediment cover was determined by counting the proportion of 100 stratified random points on the projected negative which were covered by sediment. Sediment removal was measured as the change in the percent cover of the colony.

At the completion of the experiments, the mean polyp diameter and polyp height of each colony was determined from 20 measurements. Polyp diameter is the diameter of the calice and polyp height is the vertical distance from the coenosteum to the lip of the calice. Density of the polyps (polyps/cm^2) was measured on the photographs and polyp area (the percent of the colony surface occupied by polyps) was calculated from the density and mean diameter. Measurements of the length of

the colony and its height were used to calculate the height to length ratio, a measure of the colony's convexity.

RESULTS

FIELD OBSERVATIONS

Only small amounts of sediment accumulated on living tissue in the field (Table I). Regardless of the turbidity of the water or the accumulation of sediment on the coralla, living colonies maintained essentially clear surfaces. The contrast evident in Table I between the accumulations on living tissue and their adjacent coralla

TABLE I

Dry weight of sediment collected from 25 cm^2 of *Montastrea cavernosa* substratum.

Colony*		Living tissue**	Corallum**
Galeta Is.	1 D	7.67 (1.78)	135.24 (47.13)
	2 D	11.81 (2.44)	68.23 (15.48)
	3 D	23.05 (11.40)	345.42 (81.31)
	4 D	12.69 (1.46)	183.83 (23.63)
	5 D	11.27 (3.84)	126.79 (30.22)
San Blas	1 D	11.09 (0.69)	76.93 (5.72)
	2 D	10.05 (1.15)	70.03 (4.90)
	3 D	13.09 (2.73)	93.00 (16.00)
	4 D	12.24 (1.40)	65.16 (4.46)
	5 D	9.28 (0.52)	63.67 (4.80)
	6 D	9.42 (0.70)	102.84 (39.79)
	7 N	9.61 (0.63)	66.96 (7.18)
	8 N	9.06 (0.46)	70.93 (7.71)
	9 N	10.88 (1.51)	60.11 (4.79)
	10 N	13.63 (2.48)	58.86 (4.82)

* Letter following number denotes morph designation. D, diurnal; N, nocturnal.
** Means (standard error) in mg dry weight.

demonstrates the ability of the colonies to remove sediment as rapidly as it is deposited. A possible exception to this trend is colony No. 3 at Galeta. The somewhat greater sediment accumulation observed on this colony corresponds to the exceptionally high sediment deposition on the adjacent corallum. The finding of noticeable sediment accumulation only on the colony with the greatest corallum sediment accumulation suggests the colony was operating at or near the limit of its ability to remove sediment.

Colonies at Galeta were all exposed to greater rates of sediment accumulation than in the San Blas. This is a consequence of the frequent resuspension of the muddy sands offshore of the reef at Galeta. The severity of this effect varies temporally with

sea state and spatially with position on the reef. In the 5 days of this study no significant differences were observed between days, but coralla at Galeta accumulated significantly different sediment loads ($P < 0.01$, analysis of variance, ANOVA, of data in Table I). Colonies at Galeta were located at varying heights on a small slope. Colonies 3, 4 and 5 were located within 1 m of the slope base; Colony 1 was ≈ 1 m from the base and Colony 2 about 2 m above the base. All of the colonies were situated within a single 4 × 3 m area. The coralla located at the base of the slope like Colony 3 regularly collected 0.5 cm of sediment on their surfaces. Further up the slope colonies like Colony 2 were exposed to greater amounts of current and wave action and were further removed from the muddy sands found at the base of the reef.

Less sediment deposition was observed in the San Blas. At this locality most deposition could be ascribed to fish fecal material. Physical resuspension appeared to be of minor importance.

LABORATORY EXPERIMENTS

The progress of sediment rejection as it commonly occurs is plotted in Fig. 1. Removal rates are typically high at the start of the experiment, but rapidly drop off

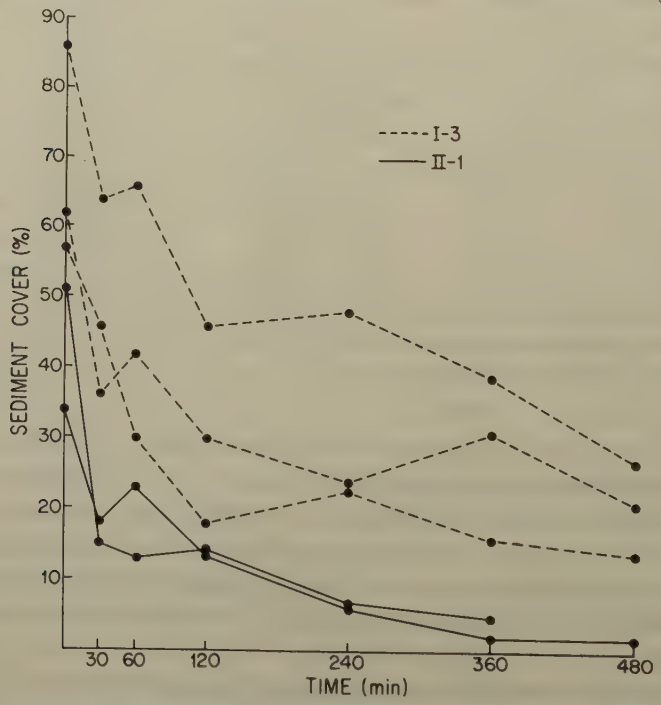

Fig. 1. Sediment removal from the surface of two colonies, II-1, a nocturnal morph, and I-3, a diurnal morph: the results of replicate experiments are represented for each colony.

as total sediment cover declines. At times the curves are reminiscent of exponential functions, but variability within and between colonies is high and the data do not fit simple exponential models. The five plots in Fig. 1 typify the between and within colony variation encountered among the 142 separate cases. Examples of both greater and lesser variance can also be found.

In order to evaluate the effects of different factors, the data for percent cover at the start of the experiment and at 120 min were examined with an ANOVA (Nie *et al.*, 1975). Morph and sediment type were analysed as treatment effects and convexity, polyp diameter, polyp height and polyp area were treated as covariates. The analysis of sediment cover after 120 min also included initial sediment cover as a covariate. Mean cover for the different treatments and the results of the analysis of variance are presented in Tables II and III.

TABLE II

Mean sediment cover (% cover ± SE) on colonies immediately after the deposition of 20 cm^3 of sediment on each colony.

	Sediment type	
	Coarse	Fine
Diurnal morph	55.5 (2.7)	31.6 (2.4)
Nocturnal morph	40.0 (3.0)	25.0 (3.4)

	Analysis of variance significance level		
Treatments	<0.001		
Morph	0.654		
Sediment	<0.001		
		simple r*	
Covariates	<0.001	fine sed.	coarse sed.
Polyp area	0.664		
Polyp height	0.009	−0.16	−0.46
Convexity	<0.001	−0.27	−0.51
Interaction	0.043		

* Product–moment correlations between initial sediment cover and the covariate. Correlations for each sediment type are presented.

The data on mean sediment cover presented in Table II show that the amount of sediment found on the test colony immediately after the experiments start varies with both the morph and sediment type. Only sediment type, however, has a significant treatment effect. The lower initial sediment loads achieved when fine sediment is used is a consequence of two factors. When the sediment is initially deposited on the colonies, the finer particles disperse more rapidly and consequently fewer actually land on the colony. Furthermore, these fine particles behave like a dense

liquid and many simply flow off of the colony surface as they land. The coarse particles have a greater tendency to "stick" where they land and flow of these particles does not occur until a sediment layer is built up.

TABLE III

Mean sediment cover (% cover ±SE) on colonies 120 min after the deposition of 20 cm^3 of sediment on each colony.

	Sediment type	
	Coarse	Fine
Diurnal morph	26.2 (2.2)	16.4 (2.0)
Nocturnal morph	18.4 (3.4)	12.2 (2.4)

	Analysis of variance significance level		
Treatments	0.109		
Morph	0.066		
Sediment	0.274		
		simple *r*	
Covariates	<0.001	fine sed.	coarse sed.
Original sed. cover	<0.001	0.71	0.64
Polyp area	0.173		
Polyp height	0.644		
Convexity	0.591		
Interaction	0.521		

The passive flow of particles off the colony is aided by colony convexity and polyp height, both of which create steeper slopes for grains to roll down. The differences between the sediment types is again evident in the simple correlation coefficients presented for the covariates. The effects of both polyp height and convexity are more pronounced when coarse sediment is used. These two covariates significantly reduce the initial sediment load of the colony.

As in nature, the nocturnal morph colonies used in this analysis exhibited greater convexity and polyp height than the diurnal morph colonies ($P = 0.05$ and $P < 0.002$, Mann–Whitney *U*-test). Consequently nocturnal morph colonies initially collect less sediment than diurnal morph colonies. Variance in initial sediment cover is, therefore, a result of differing morphologies. When considered simultaneously with the morphologic covariates no significant morph effect exists.

There is a significant sediment–morph interaction, but this may also be explained as a consequence of the differing morphologies. The ANOVA used in this analysis does not include terms for covariate-treatment interaction. Since the morphs differ morphologically, covariate–sediment effects are expressed as a morph–sediment interaction.

After 120 min sediment cover is dramatically reduced. There are no significant differences which can be ascribed to either sediment effects or morphology. The only significant factor affecting cover at this time is the initial sediment load. This indicates that colony morphology is not important in the process of sediment removal, and the slightly greater cover of the diurnal morph at this time is a consequence of greater initial cover.

These data are also suggestive of a more elaborate interpretation. At the time of initial sediment loading the differences between the morphs were quite clear and were attributable to morphologic characters. After 120 min the differences were dramatically reduced. Furthermore, significant morphologic effects are no longer in evidence and the significance of a non-morphologic, morph effect is now $P = 0.066$. This suggests there may be behavioral differences between the morphs which allow the diurnal morph to compensate for its initially greater sediment load.

The effects of daytime expansion were analysed in a third ANOVA. Diurnal morph colonies did not always keep their polyps expanded through the course of the experiment. Expansion was monitored and average polyp expansion calculated for each colony during each experiment. The third ANOVA included mean polyp expansion as a covariate and examined only diurnal morph colonies. No significant expansion effect was found.

Discussion

The fate of a sediment particle landing on a coral head can be broken into two phases. The first involves the initial impact and ends with the particle coming to rest. The second phase involves the transport of the particle off the colony.

The first phase is affected by the nature of the particle and by the morphology of the colony. The biotic response of the colony has no bearing on the amount of sediment which is initially deposited on the surface. The nature of the particle plays a great role in determining sediment load. Fine particles act like dense fluids and flow off the colony. Larger particles are more apt to remain on the colony surface. Similarly, if the polyp walls or the whole colony surface is steep (polyp height and convexity), the particle is apt to roll off.

Once a particle becomes lodged the behavioral responses of hydrostatic inflation of the polyps, ciliary action and mucus production (Hubbard & Pocock, 1972) act to remove the particle from the surface. Thompson (pers. comm.) has shown sediment rejection in both *Montastrea* species to closely follow colony wide waves of polyp inflation. Differences in these behaviors may effect different removal rates among *M. cavernosa* colonies. These differences do not significantly affect sediment cover.

Division of the sediment rejection process into two phases leads to several important conclusions. Colony morphology can have significant effects on the amount

of sediment a colony must actively transport off its surface, and the appropriate morphology can tremendously reduce active removal. Field data, however, demonstrate that under normal rates of sediment loading morphology alone will not keep *M. cavernosa* colonies sediment free, and that potentially heavy sediment accumulations are removed biotically. The magnitude of active removal estimated from the field experiments is an overestimate, since flow off bare corallum is undoubtedly less than that off living tissue. The laboratory experiments, however, illustrate the limitations of passive flow in removing sediment. Some sediment must be removed in an active process and this process compensates for initial differences introduced due to morphology.

Both morphs of *M. cavernosa* are clearly adept at keeping themselves clear of sediment. However, the morphology of the nocturnal morph removes a greater proportion of the potential sediment load through passive flow of the grains off the colonies' surface. This implies that the nocturnal morph utilizes less energy for sediment removal than the diurnal morph. However, characters like large and tall polyps, which aid in sediment removal may also have an adverse effect on the productivity of *M. cavernosa* colonies (c.f. Lasker, 1978). The net effect of the varying morphologies and behaviors is, therefore, difficult to determine.

The differences in cost and benefit of behaviors and morphology in *M. cavernosa* are probably small since the morphologic variability exhibited by this species, though impressive, is rather restricted. When the range of morphologies among all corals is considered, the energetic consequences of one morphology relative to another are probably substantial. For instance, the broad flat surfaces of *Acropora palmata* must increase the probability of a sediment particle lodging on the colony surface, and therefore increase the energetic cost of sediment rejection. In environments in which much energy is shunted into the process of sediment removal these considerations are probably important. The recognition and partitioning of morphologic and behavioral sediment rejection processes may be an important tool in the understanding of coral faunas in areas of sedimentation and resuspension.

Acknowledgements

I thank the staff and scientists of the Smithsonian Tropical Research Institute for discussion and assistance in the field. I also thank T. J. M. Schopf, R. C. Aller and J. Thompson for discussion of varying aspects of the project and two anonymous reviewers for their comments. The research was supported by NSF grant OCE 77-00148.

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J. exp. mar. Biol. Ecol., 1980, Vol. 47, pp. 89–94
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GENETICS OF SHELL COLOR IN *MYTILUS EDULIS* L. AND THE ASSOCIATION OF GROWTH RATE WITH SHELL COLOR

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Abstract: A number of crosses were made with individual mussels, *Mytilus edulis* L., which varied in shell color. The results confirm a previously published report that variation in shell color is predominately determined by a simple genetic mechanism. A study of several year classes from two separate populations shows that: (a) there is a substantial difference in the frequency of color morphs; and (b) within each population and each year class the brown morphs are 10–20% smaller than the blue morphs. These results have implications for the commercial culture of mussels.

INTRODUCTION

In spite of its name, the blue mussel, *Mytilus edulis* L., is not always blue; individuals often have brown or striped shells. This polymorphism, which is present throughout most of the range of the species, has been the subject of two recent papers. Mitton (1977) presented data showing north–south clinal variation in the color polymorphism. He reported two phenotype classes: a blue-black and a striped class, the latter in higher frequency in the south. Mitton suggested that this variation is adaptive. In the north, the darker shelled individuals in the intertidal populations will have a reduced risk of freezing, since they absorb more radiant energy. In the south, the absorption of too much sun would cause overheating and mortality; thus, the lighter phenotype would be better adapted. Innes & Haley (1977) presented data from six fullsib families which indicated that there is a genetic basis for this polymorphism. The present paper presents data from a much larger number of families which are consistent with the single locus–two-allele model as the major determinant of shell color. However, other factors appear to influence shell color as well.

MATERIAL AND METHODS

To determine the genetic basis of shell color, 2-yr-old mussels were obtained from suspended cultures of the Nova Scotia Department of Fisheries in Ostrea Lake, an inlet 50 km east of Halifax, Nova Scotia. Isolated individuals were induced to spawn by thermal stimulation. Fullsib families (one male crossed with one female) were made in the following combinations: blue male × blue female, blue male × brown female, brown male × blue female, and brown male × brown female. The

larvae in each family were grown at 15 °C in 1-l beakers. Details of the larval growth period were the same as we have previously used for oyster larvae (Newkirk *et al.*, 1977). After setting the larvae were transferred to containers with 150 μm mesh bottoms and held in trays with 16 °C flowing water for 2 months, at which time the spat had grown to 3–6 mm. The frequencies of the two color morphs were determined for each family. Shell morphs were grouped into a blue-black class (called blue) and a brown class. The former class includes all shells completely covered with blue pigment (i.e., no stripes), while the latter class includes all shells lacking blue pigment (brown) plus those that are striped. This grouping was done because there were too few striped individuals to define a separate class.

M. edulis has been cultured on rafts by the Nova Scotia Department of Fisheries for several years. Collectors (rope or plastic mesh) are put out each year just prior to natural spatfall which is concentrated in a short period during late June or early July. Analysis of size frequency distributions of these collectors in subsequent years indicates that there is very little, if any, settlement of new mussel spat in the second or third years on the established collectors. Thus, these collectors contain discrete year classes of mussels of known age and origin.

Samples from the above collectors were taken from several sources. Spat were collected at Ostrea Lake (an intermediate salinity, warm temperature area), Spanish Ship Bay (a high salinity, low temperature area), and some spat from Ostrea Lake had been transferred in the fall of 1975 and 1976 to another site, Whitehead (a cold, high salinity area). The frequency of color morphs was determined for each sample, and the shell height of a sample of individuals in each morph class was recorded.

Results

There is a clear pattern in the frequencies of the color morphs that occurred in the offspring from the various crosses (Table I). The averages by type of cross are shown in Table I. All offspring from the crosses between a blue male and blue female were blue. The crosses between a brown male and a blue female produced offspring with an average frequency of 60% blue shells. The offspring of a blue male and a brown female had an average frequency of 56% blue shells. Combining all the crosses between a blue parent and a brown parent gives an average frequency of 58%. The last type of cross, between a brown male and a brown female, produced offspring with an average frequency of 37% blue shells.

The data on shell color frequency and mean shell height of the color morphs are given in Table II. The frequency of blue shells was 90% or greater in all three year classes taken from Ostrea Lake, while at Spanish Ship Bay the frequency was 50%. In every sample the blue individuals had a mean shell height larger than the brown individuals. The percentage difference varied from 11 to 21%, and in every case the difference was statistically significant.

TABLE I

Number of offspring of each color morph and the percentage that were blue in the matings between known parents.

	Blue female			Brown female		
	Blue	Brown	% Blue	Blue	Brown	% Blue
Blue male	4	0	100	0	5	0
	6	0	100	16	8	67
	20	0	100	31	35	47
	70	0	100	12	2	86
	45	0	100	9	6	60
	82	0	100	7	12	37
	91	0	100	45	27	62
	19	0	100	62	47	57
	40	0	100	27	7	79
				7	5	64
	Average		100	Average		60
Brown male	14	0	100	3	3	50
	1	2	33	19	49	28
	2	1	67	6	8	43
	67	54	55	42	83	34
	3	3	50	15	50	23
	13	11	54	16	34	32
				14	13	52
	Average		56	Average		37

TABLE II

Average length of mussels of different colors from both Ostrea Lake (O.L.) and Spanish Ship Bay (S.S.B.) grown at O.L., S.S.B. and Whitehead (W.H.); data given are length in cm, number measured (n), standard deviation of length (s), t-test of difference in mean length of color morphs (t) with the degrees of freedom (d.f.), and the percentage of each color morph in the population (%) with the number (n') used to determine the percentage.

Source of spat	Year	Growing site	Date measured	Color	Length (cm)	n	s	t	d.f.	%	n'
O.L.	1977	O.L.	Oct. '77	blue	1.39	68	0.32	2.05	89	96.4	616
				brown	1.24	23	0.30			3.6	23
O.L.	1976	W.H.	Nov. '78	blue	3.43	208	0.87	2.33	261	98.1	208
				brown	3.10	55	0.95			1.9	4
O.L.	1975	W.H.	Nov. '78	blue	4.85	185	1.14	5.29	229	90.1	182
				brown	4.02	46	0.90			9.9	20
S.S.B.	1977	S.S.B.	Nov. '77	blue	0.64	107	0.17	3.93	321	50.6	221
				brown	0.56	216	0.17			49.4	216
S.S.B.	1976	S.S.B.	Nov. '77	blue	2.24	151	0.76	3.64	295	50.8	151
				brown	1.94	146	0.66			48.2	146

Discussion

The data presented here confirm the earlier results of Innes & Haley (1977) which showed a genetic control of the shell color in *M. edulis*. However, the genetic mechanism may not be as simple as the proposed single locus–two-allele mechanism, with one allele dominant and the other recessive for blue. The present results support the hypothesis of a recessive genotype producing a blue phenotype since all crosses between two blue parents resulted in all blue offspring, while any other type of cross resulted in a mixture. According to this model, single crosses between a blue parent and a brown parent will be crosses between the recessive homozygote and either the heterozygote or the dominant homozygote. In such a case, the offspring will be expected to be either 50% or 0% blue, respectively. Present results gave frequencies from 100 to 0% with an average of 58% blue, very close to 50% as might be expected if these were all crosses of homozygote recessive and heterozygotes. The fact that none of the adults appear to be homozygous dominant is not surprising since the year class from which these animals were taken has a frequency of brown shells of 5%. The brown individuals used here were selected out because of their color. With such a low frequency of the brown allele, the dominant homozygotes have an expected frequency of 0.25% or less in the population or 2.6% or less among the brown phenotypes.

The single locus–two-allele model would predict that the offspring of brown crossed with brown would be either 25% blue or all brown, corresponding to the cross of two heterozygotes or the dominant homozygote with either a heterozygote or another homozygote. As indicated in the crosses with blue adults, none of the brown adults appeared to be a dominant homozygote since all parents had some offspring that were blue, i.e., homozygous recessive. The observed results ranged in frequency from 23 to 52%, with an average of 37%. This is higher than the 25% expected from crosses of two heterozygotes.

The discrepancies from the genetic model of Innes & Haley (1977) that were observed here are in the form of more blue offspring from both the brown × brown crosses and the blue × brown crosses. The excess of blue offspring in each of these groups is significant using a χ^2 test, $P < 0.05$. There are several possible reasons for these discrepancies: first, selection of the genotypes before the stage at which the color frequencies were observed, or secondly, genotype-environment interaction. In many of the crosses, the survival of the larvae and the spat was sufficiently low that selective mortality could easily account for the apparent excess of blue individuals.

A third possible explanation of the deviations from the expected frequencies could be the presence of modifier loci. The observed phenotypes have been classified as blue or brown for simplicity. In fact, as pointed out earlier, the brown group is not homogeneous. There are some individuals with different intensities of the blue pigment in the shell resulting in shells that vary from light to dark brown. There is

also the presence of blue stripes in some "brown" phenotypes in the population. The crosses that have been made so far have not been extensive enough to determine whether these differences have a genetic component, but it is possible that there are modifier loci which interact with the major locus, resulting in a deviation from expected frequencies.

Although there is evidently a major genetic component to the determination of shell color in *M. edulis*, there may also be an environmental influence which could have affected the observed frequencies. There are a few observations that indicate an occasional nongenetic component in the expression of shell color. Very rarely have mussels 2 to 3 yr old (grown in suspended culture), been observed to change color abruptly. If they do, it is occasionally coincidental with the annual rings but, more often, it is a gradual change after the first year. All observed changes have been from brown to blue. The other observation that suggests an environmental component is that occasionally the striped pattern of the left and right shell will not be exactly the same. The overall pattern will be similar, e.g., all fine stripes or a few wide stripes, but the position and width of the stripes may vary. This suggests a developmental change has occurred at some point to change the location or action of the sites in the mantle edge that deposit the pigment in the shell.

At present, it is concluded that the major determinant of shell color variation in *M. edulis* is under a relatively simple genetic mechanism. The data are, for the most part, consistent with the single locus model proposed by Innis & Haley (1977). However, there may be more than one dominant brown allele which have slightly different phenotypic expressions in association with the recessive blue allele. There may also be one or more additional loci influencing shell color to a lesser extent, perhaps one causing the striping.

A substantial difference is evident between the two populations studied in the frequency of color morphs. The difference is larger than the maximum range in frequencies reported by Mitton (1977) from Maine to Virginia. Using the genetic model discussed above, the phenotype frequencies observed correspond to a frequency of the homozygous recessive genotype (blue) of about 50% at Spanish Ship Bay and at least 90% at Ostrea Lake or gene frequencies of the recessive allele of 0.71 and 0.95 (or more), respectively, the higher temperature environment (Ostrea Lake) having the higher frequency of blue phenotypes. It is unlikely that the environment has much direct influence in modifying the expression of the genotype of the spat or juveniles. The two samples of Ostrea Lake spat grown at Whitehead show the same high frequency of blue shells, and experimental transplants from Ostrea Lake to Spanish Ship Bay have not shown a change in color morph frequencies.

The association of color frequency and temperature in the data presented here is opposite the geographic trend Mitton (1977) observed, but the association with growth rate holds even when the mussels are transplanted from the warmer environment to a colder one (i.e., Ostrea Lake to Whitehead). The physiological

mechanisms and the interaction with environmental parameters clearly need to be examined more closely.

The observation of a correlation between shell color and growth rate indicates a physiological link between the gene or genes determining shell color and growth rate. The primary role of this gene may be metabolic, and shell color may be an ancillary expression of the gene products.

Differences in growth rate are independent of the differences in light absorption of the color morphs as these animals were grown in suspended culture and at ambient seawater temperature. Thus the postulated selective mechanism of Mitton would not apply here. It remains to be seen whether the geographic cline in color morphs observed by Mitton is a direct result of the solar radiation as postulated or whether it is a result of the correlated effects of shell color and physiological parameters as observed here. The clear association of mean growth rate and shell color suggests more than a simple color-latitude adaptation.

Waldron *et al.* (1976) reported population differences in a polymorphism of *M. edulis* foot pigmentation. The differences in color frequencies occurred over short distances (as little as 30 m) and were associated with habitat differences. Their study was done on wild populations; thus it did not give any indication of a possible genetic basis. They suggested that these differences were the result of selection, but it is unclear how this would act in a polymorphism where the color differences are not useful in camouflage as in the case of *Crepidula* spp. (Hoagland, 1977).

The above results have implications for those who are culturing mussels. If the correlation of color and growth rate and population differentiation by color are general phenomena, the sources of spat for culture may be critical. But it must be emphasized that before shell color becomes a key for spat collection, the association of color and growth rate should be established in the populations concerned.

Acknowledgements

I am grateful to Dr. T. McKay for comments on an earlier draft of this paper and to the Nova Scotia Department of Fisheries for support and cooperation during this study.

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PREDATION, ATTACK SUCCESS, AND ATTRACTION TO THE BAY SCALLOP, *ARGOPECTEN IRRADIANS* (Lamarck) BY THE OYSTER DRILL, *UROSALPINX CINEREA* (Say)

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Abstract: Investigation of the intensity of predation by the oyster drill, *Urosalpinx cinerea* (Say), on the bay scallop, *Argopecten irradians* (Lamarck), was carried out in field and laboratory studies. Field measurements of densities of the oyster drill and the bay scallop were respectively, $3.6 \cdot m^{-2}$, and $21.1 \cdot m^{-2}$ on a scallop bed in Ninigret Pond, Charlestown, Rhode Island. The mean density of snails attacking scallops was $1.7 \cdot m^{-2}$. In a laboratory study of attack success, an average of 72.3% of drill attacks led to death of the scallop. Snails were also shown to be strongly attracted to scallop effluent in a choice chamber.

INTRODUCTION

Barnacles, oysters, and mussels are often listed as primary prey for the oyster drill, *Urosalpinx cinerea* (Wood, 1968; Carriker & Van Zandt, 1972). It is also known that drills are guided by olfactory cues to their common prey (Wood, 1968; Pratt, 1974) but evidence that drills attack scallops is scarce, although Marshall (1960) found that many *U. cinerea* were attracted to caged bay scallops in a Connecticut estuary. Our purpose was to determine the importance of *U. cinerea* (Say) as a predator of the bay scallop *Argopecten irradians* (Lamarck), on a natural scallop bed, including drill attack success and the effect of scallop effluent on oyster drills in a choice chamber.

METHODS AND RESULTS

FIELD STUDY

Methods

Field studies were carried out using SCUBA during July 1979 on a natural scallop bed in Ninigret Pond, Charlestown, Rhode Island. Square meter quadrats were randomly placed to measure scallop and oyster drill densities as well as the density of drill attacks. Attacks were defined as single or multiple occurrences of *Urosalpinx cinerea* attached to a live scallop. Densities were measured by removing all scallops and oyster drills from a m^2 quadrat after noting the number of drill

attacks. Counts for each m^2 were made at the surface. Animals touching the square meter frame were considered to be within the quadrat. Mean densities were estimated from 21-m^2 quadrats. Temperature measurements ranged from 23–26 °C, while salinity was constant at 28‰. Scallops and drills collected during this phase were held in ambient running sea water for subsequent laboratory studies.

Results

The mean (±2 SE) scallop density on a scallop bed in Ninigret Pond was $21.2 \pm 2.3 \cdot m^{-2}$, while the density of oyster drills was $3.6 \pm 1.2 \cdot m^{-2}$. No other major predators, including the starfish *Asterias forbesi* (Desor), were observed during data collection. Although one blue crab, *Callinectes sapidus* (Rathbun), was found eating scallop remains, there was no evidence to show that the crab actually killed the scallop. The mean (±2 SE) density of attacks by *Urosalpinx cinerea* was $1.7 \pm 0.6 \cdot m^{-2}$. Our calculations show that 7.9% of the scallops on this bed were being attacked by 47% of the drill population and that 6.8% of these scallops were attacked by more than one drill.

LABORATORY ASSESSMENT OF OYSTER DRILL ATTACK SUCCESS

Methods

To examine attack success, drills and scallops collected from Ninigret Pond were placed in a tank (118 × 240 × 27 cm), with a bottom area of 2.8 m^2. Only drills measuring 1.5 – 2 cm from apex to siphon were used. Prior to testing, all drills were provided with liberal numbers of live scallops on which to feed. After 24 h, each scallop with a drill on its shell was isolated in a 40-l tank with running sea water where the fate of each scallop could be monitored. Attacks were considered unsuccessful when a snail was found off the scallop and successful if examination of a dead scallop revealed a completed borehole. Dead scallops with boreholes were assumed to have died directly or indirectly as a result of a snail attack. This procedure was replicated three times with different snails at a mean (±2 SE) water temperature of 19.9 ± 0.8 °C and a mean (±2 SE) salinity of 30.0 ± 0.4‰ during August and September 1979.

Results

Of the attacking oyster drills, an average ($\bar{x}$ ±2 SE) of 72.3 ± 9.8% succeeded with their attacks culminating in scallop death while only an average ($\bar{x}$ ±2 SE) of 25.9 ± 12.2% of scallops survived attacks. Results of the three replicates are displayed in Table I. Although both replicates performed at 20 °C appear to be the same, attack success of snails at 17.7 °C tends to be lower even though it is not significantly different (χ^2). The mean time for success is similar at all temperatures,

TABLE I

Laboratory assessment of the percentage of successful attacks by the oyster drill, *Urosalpinx cinerea*, on the bay scallop, *Argopecten irradians*, and mean time required to complete the attack.

	n	Successful attacks (%)	Unsuccessful attacks (%)	$\bar{X} \pm 2$ SE days until scallop death	$\bar{X} \pm 2$ SE temp. (°C)
Replicate 1 22 Aug.–5 Sept.	18	77.8	16.7	6.1 ± 1.5	20.9 ± 0.8
Replicate 2 29 Aug.–4 Sept.	17	76.5	23.5	5.9 ± 1.3	20.6 ± 0.8
Replicate 3 20 Aug.–1 Oct.	16	62.5	37.5	6.3 ± 2.1	17.7 ± 0.7
		$\bar{X} = 72.3$ 2 SE = 9.8	$\bar{X} = 25.9$ 2 SE = 12.2	$\bar{X} = 6.1 \pm 0.9$	$\bar{X} = 19.9 \pm 0.7$

averaging 6.1 days (range: 2–15 days) with the majority of successful attacks occurring between Day 4 and Day 7.

OYSTER DRILL RESPONSE TO SCALLOP EFFLUENT

Methods

Scallops and oyster drills collected as previously described from Ninigret Pond were held in ambient temperature, unfiltered, running sea water for 2 wk prior to experimentation. Oyster drills were supplied with live scallops for food. During the experiments, temperatures and salinities averaged ($\bar{x} \pm 2$ SE) 21.3 ± 2.0 °C and 30.0 ± 0.4‰ respectively.

Trials were carried out with epoxy-coated plywood choice chambers designed by Pratt (1974). Continuously running sea water was first fed into two reservoirs ($21 \times 19 \times 22$ cm) from which the water was gravity fed into wells on both sides of the choice chamber. During tests, scallop effluent was introduced into sea water by placing 30 live scallops into one of the reservoirs. Water from the wells overflowed down both ramps a distance of 19.5 cm to exit through holes in a drain plate at the center of the apparatus. Flow rates to the wells were set with valves at 250 ml $\cdot$ min^{-1}. Dye experiments showed that mixing occurred only in a limited area over the drain plate holes. To eliminate possible directional bias, scallop effluent was presented on different sides of the choice chamber after six drills were tested.

Trials were performed by starting three marked snails directly in the center of the drain plate with their long axes perpendicular to the chamber length. After 1 h the distance in cm each drill travelled away from the start was measured from the center of the chamber to the tip of the drill siphon. Drills were replaced with new snails after they responded or if they failed to move from the starting position after 1 h. Although Wood (1968) found that *U. cinerea* do not follow each others trails, as a precautionary measure ramps were scrubbed after every six drills tested with

sea water during each trial. If a snail reached the well in less than 1 h, both distance and time in minutes were recorded. During the first of two trials, 88 different drills were presented with sea water on both sides of the chamber to test for chamber bias. In the second trial, 100 different drills were presented with a choice between scallop effluent and "plain" sea water to measure drill response to scallops. Data were analyzed using the χ^2 and *t*-test.

Results

When presented with a choice between plain sea water versus plain sea water in Trial 1 (Table II), the number of *U. cinerea* responding and moving greater than halfway up the ramp (10 cm) was the same for both sides of the chamber (χ^2). Measurements of the distance travelled and the rate that drills moved up either ramp showed no difference between sides further indicating that there was no significant apparatus bias (*t*-test).

Responses of drills presented with a choice between sea water containing scallop

TABLE II

Responses of *Urosalpinx cinerea* to bay scallop effluent and plain sea water in the choice chamber.

	Trial 1 ($n = 88$)		Significance			
Water presented	Plain sea water	Plain sea water	χ^2	*t*-test	d.f.	*P*
Number responding	44	44	0.0	—	1	0.99
Number moving ≧10 cm	4	6	0.4	—	1	<0.1
$\bar{X} \pm 2$ SE cm travelled	2.6 ± 1.4	3.8 ± 1.8	—	1.04	86	>0.2
$\bar{X} \pm 2$ SE rate of movement (cm/min)	0.08 ± 0.07	0.09 ± 0.05	—	0.22	86	>0.5
	Trial 2 ($n = 100$)					
	Scallop effluent	Plain sea water				
Number responding	65	35	9.0	—	1	<0.005
Number moving ≧10 cm	33	7	16.7	—	1	<0.005
$\bar{X} \pm 2$ SE cm travelled	10.8 ± 2.0	5.4 ± 2.2	—	3.36	98	<0.001
$\bar{X} \pm 2$ SE rate of movement (cm/min)	0.35 ± 0.1	0.17 ± 0.11	—	2.19	98	<0.05

effluent and plain sea water in Trial 2 (Table II) showed a significant number of snails responded to scallop effluent compared to those responding to plain sea water (χ^2). The number of snails moving > 10 cm was also greater in water containing scallop effluent (χ^2). Further, drills travelled twice the distance and speed in scallop effluent sea water compared to distances travelled in plain sea water.

Discussion

U. cinerea is an active predator of *Argopecten irradians* in Ninigret Pond, Rhode Island and was the only predator found during our observations. Although other predators have been listed as preying on scallops (Marshall, 1960), we have no data on their actual or potential impact in Ninigret Pond. During the spring, when water temperatures were 10 °C, we observed *Asterias forbesi* preying on scallops in the study area. However, starfish were conspicuously absent from our summer observations, possibly owing to their inability to cope with temperatures above 25 °C (Mackenzie, 1969). Further, our observations on laboratory-held starfish confirm that mortalities were unusually high when ambient water temperature was above 20 °C. *Urosalpinx cinerea*, unlike starfish, feeds at a maximum rate (Hanks, 1957; Manzi, 1970) at the salinities and temperatures encountered during our observations.

Our laboratory studies show that not all drill attacks reach a successful conclusion. More than one-quarter of all drill attacks on scallops are not successful and may be a direct result of the vigorous escape response (Ordzie & Garofalo, 1980) which bay scallops employ when attacked. We observed that the rapid movements of the scallop valves during this response were sufficient to shake off an attached drill. Furthermore, live scallops in the field were found with complete and incomplete boreholes. Calculations of predation by *U. cinerea* on scallops based only on observed attacks without correction for unsuccessful attacks would lead to an overestimation of predation intensity.

Measurements of the time required to successfully drill scallops are in agreement with rates for *U. cinerea* to drill oysters (Carriker & Van Zandt, 1972), and are similar to those found for *Ocinebra japonica* (Chew, 1960).

Environmental parameters have such strong influences on feeding rates of predatory snails (Menge, 1978) that predation rates differ between habitats and even between individuals. Such observations make it difficult to generalize about predation rates. The impact of predation is very specific for habitat location and environmental conditions.

We have also shown that *Urosalpinx cinerea* move toward water containing scallop effluent. This strong attraction was heretofore only observed when drills were presented with barnacle effluent and is unusually strong compared to other bivalve species tested, including oysters (Wood, 1968; Pratt, 1974). Furthermore, once stimulated, oyster drills proceed to double their rate of locomotion toward the

scallops. Moving faster toward prey effluent increases the probability of prey encounter in a shorter time period. By decreasing the time between recognition of prey effluent and contact with mobile prey, drills also increase the probability of being able to execute an attack before the prey changed location.

Acknowledgements

This research was funded by the Environmental Control Division, U.S. Department of Energy under Contract No. E (11–1)–4047. We thank the Rhode Island Department of Environmental Management for use of the laboratory facility. We also express our thanks to A. Ganz, K. Goggin, W. Lapin, and B. Polonetz for their help during the field studies. This paper represents contribution No. 1916 of the Rhode Island Agricultural Experiment Station.

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